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[ ] continuation patent application of
[ ] divisional patent application of
[X] continuation-in-part patent application of

Inventor(s)/Applicant Identifier: **John Fikes, Alessandro Sette, John Sidney, Scott Southwood, Robert Chesnut, Esteban Celis and Elissa Keogh**

For: **INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS**

[X] This application claims priority from each of the following Application Nos./filing dates: 09/189,702 filed November 10, 1998; 08/205,713 filed March 4, 1994; 08/159,184 filed November 29, 1993; 08/073,205 filed June 4, 1993 and 08/027,146 filed March 5, 1993, the disclosure(s) of which is (are) incorporated by reference.

[ ] Please amend this application by adding the following before the first sentence: "This application is a [ ] continuation [ ] continuation-in-part of and claims the benefit of U.S. Application No. 60/_____, filed _____, the disclosure of which is incorporated by reference."

Enclosed are:

[X] 181 page(s) of specification
[X] 6 page(s) of claims
[X] 1 page of Abstract
[ ] _____ sheet(s) of [ ] formal [ ] informal drawing(s).
[ ] An assignment of the invention to _____
[X] A [ ] signed [ ] unsigned Declaration & Power of Attorney
[ ] A [ ] signed [ ] unsigned Declaration.
[ ] A Power of Attorney.
[ ] A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27 [ ] is enclosed [ ] was filed in the prior application and small entity status is still proper and desired.
[ ] A certified copy of a _____ application.
[ ] Information Disclosure Statement under 37 CFR 1.97.
[ ] A petition to extend time to respond in the parent application.
[ ] Notification of change of [ ] power of attorney [ ] correspondence address filed in prior application.
[ ]

In view of the Unsigned Declaration as filed with this application and pursuant to 37 CFR §1.53(f), Applicant requests deferral of the filing fee until submission of the Missing Parts of Application.

DO NOT CHARGE THE FILING FEE AT THIS TIME.

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PATENT APPLICATION

INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

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PATENT

Attorney Docket No.: 018623-014600US

INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a Continuation-In-Part ("CIP") of U.S.S.N. 09/189,702, filed 11/10/98, which is a CIP of U.S.S.N 08/205,713 filed 3/4/94, which is a CIP of abandoned U.S.S.N. 08/159,184 filed 11/29/93, which is a CIP of abandoned U.S.S.N. 08/073,205 filed 6/4/93 which is a CIP of abandoned U.S.S.N 08/027,146 filed 3/5/93. The present application is also related to U.S.S.N. 09/226,775, which is a CIP of abandoned U.S.S.N. 08/815,396, which claims benefit of abandoned U.S.S.N. 60/013,113. Furthermore, the present application is related to U.S.S.N. 09/017,735, which is a CIP of abandoned U.S.S.N. 08/589,108; U.S.S.N. 08/454,033; and U.S.S.N. 08/349,177. The present application is also related to U.S.S.N. 09/017,524, U.S.S.N. 08/821,739, which claims benefit of abandoned U.S.S.N. 60/013,833; and U.S.S.N. 08/347,610, which is a CIP of U.S.S.N. 08/159,339, which is a CIP of abandoned U.S.S.N. 08/103,396, which is a CIP of abandoned U.S.S.N. 08/027,746, which is a CIP of abandoned U.S.S.N. 07/926,666. The present application is also related to U.S.S.N. 09/017,743, which is a CIP of abandoned U.S.S.N. 08/590,298; and U.S.S.N. 08/452,843, which is a CIP of U.S.S.N. 08/344,824, which is a CIP of abandoned U.S.S.N. 08/278,634. The present application is also related to PCT application 99/12066 filed 5/28/99 which claims benefit of provisional U.S.S.N. 60/087,192, and U.S.S.N. 09/009,953, which is a CIP of abandoned U.S.S.N. 60/036,713 and abandoned U.S.S.N. 60/037,432. In addition, the present application is related to U.S.S.N. 09/098,584, U.S.S.N. 09/239,043, U.S.S.N. 60/117,486, U.S.S.N. 09/350,401, and U.S.S.N. 09/357,737. In addition, the present application is related to U.S. Patent Application entitled "Inducing Cellular Immune Responses to p53 Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-014500, filed of even date herewith; U.S. Patent Application entitled "Inducing Cellular Immune Responses to Carcinoembryonic Antigen Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-014400, filed of even date herewith; and U.S. Patent Application entitled "Inducing Cellular Immune Responses to HER2/neu Using Peptide and Nucleic Acid Compositions", Attorney Docket No. 018623-

014800, filed of even date herewith. All of the above applications are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was funded, in part, by the United States government under grants with the National Institutes of Health. The U.S. government has certain rights in this invention.

INDEX

I. BACKGROUND OF THE INVENTION

A growing body of evidence suggests that cytotoxic T lymphocytes (CTL) are important in the immune response to tumor cells. CTL recognize peptide epitopes in the context of HLA class I molecules that are expressed on the surface of almost all nucleated cells. Following intracellular processing of endogenously synthesized tumor antigens, antigen-derived peptide epitopes bind to class I HLA molecules in the endoplasmic reticulum, and the resulting complex is then transported to the cell surface. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms, *e.g.*, activation of lymphokines such as tumor necrosis factor-α (TNF- α) or interferon-γ (IFNγ) which enhance the immune response and facilitate the destruction of the tumor cell.

Tumor-specific helper T lymphocytes (HTLs) are also known to be important for maintaining effective antitumor immunity. Their role in antitumor immunity has been demonstrated in animal models in which these cells not only serve to provide help for induction of CTL and antibody responses, but also provide effector functions, which are mediated by direct cell contact and also by secretion of lymphokines (*e.g.*, IFNγ and TNF- α).

A fundamental challenge in the development of an efficacious tumor vaccine is immune suppression or tolerance that can occur. There is therefore a need to establish vaccine embodiments that elicit immune responses of sufficient breadth and vigor to prevent progression and/or clear the tumor.

The epitope approach, as we have described, may represent a solution to this challenge, in that it allows the incorporation of various antibody, CTL and HTL epitopes, from discrete regions of a target TAA, and/or regions of other TAAs, in a single vaccine composition. Such a composition may simultaneously target multiple dominant and subdominant epitopes and thereby be used to achieve effective immunization in a diverse population.

MAGE, melanoma antigen genes, are a family of related proteins that were first described in 1991. Van der Bruggen and co-workers identified the MAGE gene after isolating CTLs from a patient who demonstrated spontaneous tumor regression. These CTLs recognized melanoma cell lines as well as tumor lines from other patient all of whom expressed the same HLA-A1-restricted gene (van der Bruggen *et al.*, *Science*

254:1643-1647, 1991; DePlaen *et al.*, *Immunogenetics* 40:360-369, 1994). The MAGE genes are expressed in metastatic melanomas (*see, e.g.*, Brasseur *et al.*, *Int. J. Cancer* 63:375-380, 1995), non-small lung (Weynants *et al.*, *Int. J. Cancer* 56:826-829, 1994), gastric (Inoue *et al.*, *Gastroenterology* 109:1522-1525, 1995), hepatocellular (Chen *et al.*, *Liver* 19:110-114, 1999), renal (Yamanaka *et al.*, *Human Pathol.* 24:1127-1134, 1998), colorectal (Mori *et al.*, *Ann. Surg.* 224:183-188, 1996), and esophageal (Quillien *et al.*, *Anticancer Res.* 17:387-391, 1997) carcinomas as well as tumors of the head and neck (Lett *et al.*, *Acta Otolaryngol.* 116:633-639, 1996), ovaries (Gillespie *et al.*, *Br J. Cancer* 78:816-821, 1998; Yamada *et al.*, *Int. J. Cancer* 64:388-393, 1995), bladder, and osteosarcoma (Sudo *et al.*, *J. Orthop. Res.* 15:128-132, 1997). Thus, MAGE2/3 are important targets for cancer immunotherapy.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards TAAs. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. For example, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines. Such immunosuppressive epitopes may, *e.g.*, correspond to immunodominant epitopes in whole antigens, which may be avoided by selecting peptide epitopes from non-dominant regions (*see, e.g.*, Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996).

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune

response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen (a "pathogen" may be an infectious agent or a tumor associated molecule). Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from the pathogen in a vaccine composition.

Furthermore, an epitope-based anti-tumor vaccine also provides the opportunity to combine epitopes derived from multiple tumor-associated molecules. This capability can therefore address the problem of tumor-to tumor variability that arises when developing a broadly targeted anti-tumor vaccine for a given tumor type and can also reduce the likelihood of tumor escape due to antigen loss. For example, a melanoma in one patient may express a target TAA that differs from a melanoma in another patient. Epitopes derived from multiple TAAs can be included in a polyepitopic vaccine that will target both melanomas.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele. Impractically large numbers of epitopes would therefore have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA molecules do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse

segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500 nM or less for HLA class I molecules or an IC_{50} of 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes embodiments comprising methods for monitoring or evaluating an immune response to a TAA in a patient having a known HLA-type. Such methods comprise incubating a T lymphocyte sample from the patient with a peptide composition comprising a TAA epitope that has an amino acid sequence described in Tables VII to Table XX or Table XXII which binds the product of at least one HLA allele present in the patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope may, for example, be used as a component of a tetrameric complex for this type of analysis.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to the pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

not applicable

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to a TAA by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to the TAA. The complete sequence of the TAA proteins to be analyzed can be obtained from GenBank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of particular TAAs, as will be clear from the disclosure provided below.

A list of target TAA includes, but is not limited to, the following antigens: MAGE 1, MAGE 2, MAGE 3, MAGE-11, MAGE-A10, BAGE, GAGE, RAGE, MAGE-C1, LAGE-1, CAG-3, DAM, MUC1, MUC2, MUC18, NY-ESO-1, MUM-1, CDK4, BRCA2, NY-LU-1, NY-LU-7, NY-LU-12, CASP8, RAS, KIAA-2-5, SCCs, p53, p73, CEA, Her 2/neu, Melan-A, gp100, tyrosinase, TRP2, gp75/TRP1, kallikrein, PSM, PAP, PSA, PT1-1, B-catenin, PRAME, Telomerase, FAK, cyclin D1 protein, NOEY2, EGF-R, SART-1, CAPB, HPVE7, p15, Folate receptor CDC27, PAGE-1, and PAGE-4.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity. Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or

a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see*, *e.g.*, Stites, *et al.*, IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a

reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 19990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50}, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a

specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues,

preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and/or physiologically compatible composition.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or

intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is not obtained from natural sources, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. Symbols for the amino acids are shown below.

Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to a TAA in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al., Cell* 47:1071, 1986; Babbitt, B. P. *et al., Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see* also, *e.g.*, Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via web at : http://134.2.96.221/scripts.hlaserver.dll/home.htm; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics*, in press, 1999).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See*, *e.g.*, Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.*, Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide sensitized target cells.

2) Immunization of HLA transgenic mice (*see, e.g.*, Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (*see, e.g.*, Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response "naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is $\leq$ 500 nM). HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq$ 1,000 nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

As disclosed herein, higher HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. Moreover, higher binding affinity peptides lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al., J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold

range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g.,* Schaeffer *et al., Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g.*, Southwood *et al. J. Immunology* 160:3363-3373,1998, and co-pending U.S.S.N. 09/009,953 filed 1/21/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets endogenously expressing the epitope exhibit binding affinity or IC_{50} values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were obtained. CTL inductions positive for peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques will identify about 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may

represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.*, Tables I-III), or if the presence of the motif corresponds to the ability to bind several allele-specific HLA molecules, a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC_{50} by using the following formula: IC_{50} of the standard peptide/ratio = IC_{50} of the test peptide (*i.e.*, the peptide epitope). The IC_{50} values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC_{50} values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assays described herein are examples of standards; alternative standard peptides can also be used when performing binding studies.

To obtain the peptide epitope sequences listed in each Table, protein sequence data for MAGE2/3 were evaluated for the presence of the designated supermotif or motif. The "pos" (position) column in the Tables designates the amino acid position in the MAGE2/3 proteins that corresponds to the first amino acid residue of the putative epitope. The "number of amino acids" indicates the number of residues in the epitope sequence. Preferred epitopes are those that are conserved between the MAGE2 and MAGE3 sequences. The "A" and "B" designations on the Tables refer to MAGE2 and MAGE3, respectively.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI. In some cases, peptide epitopes may be listed in both a motif and a supermotif Table. The relationship of a particular motif and respective supermotif is indicated in the description of the individual motifs.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least: A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992; Ruppert *et al.*, *Cell* 74:929-937, 1993) and cross-reactive binding among HLA-A2 and -A28 molecules have been described. (*See, e.g.*, Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994 for reviews of relevant data.) These primary anchor

residues define the HLA-A2 supermotif; which presence in peptide ligands corresponds to the ability to bind several different HLA-A2 and -A28 molecules. The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope, *e.g.*, in position 9 of 9-mers (*see, e.g.*, Sidney *et al.*, *Hum. Immunol.* 45:79, 1996). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sette and Sidney, *Immunogenetics*, in press, 1999). The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least: A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins comprising at least: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.,* Sidney, *et al., J. Immunol.* 154:247, 1995; Barber, *et al., Curr. Biol.* 5:179, 1995; Hill, *et al., Nature* 360:434, 1992; Rammensee, *et al., Immunogenetics* 41:178, 1995 for reviews of relevant data). Other allele-specific HLA molecules predicted to be members of the B7 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B7 supermotif are set forth on the attached Table XI.

IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999). Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney et al., *Immunol. Today* 17:261, 1996). Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4404. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999 for reviews of relevant data). Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific

HLA molecules predicted to be members of the B58 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B58 supermotif are set forth on the attached Table XIII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999). Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, J. Immunol., 152:620, 1994; Kondo *et al.*, *Immunogenetics* 45:249, 1997; and Kubo *et al.*, *J. Immunol.* 152:3913, 1994 for reviews of relevant data). Peptide binding to HLA-A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII, as these residues are a subset of the A1 supermotif primary anchors.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991) and was further found to comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (*see, e.g.*, Hunt *et al.*, Science 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). The A*0201 allele-specific motif has also been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M or T as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (*see, e.g.*, Ruppert *et al.*, *Cell* 74:929-937, 1993). These are shown in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A*0201 motif are set forth on the attached Table VIII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, sY, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, *Proc. Natl. Acad. Sci USA* 90:1508, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the attached Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX. The A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele specific motif primary anchor residues.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 90:2217-2221, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, *Kondo et al.*, *J. Immunol.* 155:4307-4312, 1995; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or

secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes, as the primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701 (*see, e.g.*, the review by Southwood *et al. J. Immunology* 160:3363-3373,1998). Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified (Southwood *et al., supra*). These are set forth in Table III. Peptide binding to HLA-DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Potential epitope 9-mer core regions comprising the DR-1-4-7 supermotif, wherein position 1 of the supermotif is at position 1 of the nine-residue core, are set forth in Table XIX. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a c nine residue core, are also shown in the Table, along with cross-reactive binding data for the exemplary 15-residue supermotif-bearing peptides.

IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules (*see, e.g.*, Geluk *et al.*, *J. Immunol.* 152:5742, 1994). In the first motif (submotif DR3a) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3b): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Potential peptide epitope 9-mer core regions corresponding to a nine residue sequence comprising the DR3a submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a nine residue core, are also shown in Table XXa along with binding data of the exemplary DR3 submotif a-bearing peptides.

Potential peptide epitope 9-mer core regions comprising the DR3b submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-b epitope are set forth in Table XXb. Binding data of exemplary DR3 submotif b-bearing peptides is also shown.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid

compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are each present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups. The incremental coverage obtained by the inclusion of A1,- A24-, and B44-supertypes to the A2, A3, and B7 coverage and coverage obtained with all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al., Adv. Immunol.* 27:5159, 1979; Bennink, *et al., J. Exp. Med.* 168:19351939, 1988; Rawle, *et al., J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al., Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al., J. Immunol.* 131:1635, 1983); Rosenthal, *et al., Nature* 267:156-

158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

Because tissue specific and developmental TAAs are expressed on normal tissue at least at some point in time or location within the body, it may be expected that T cells to them, particularly dominant epitopes, are eliminated during immunological surveillance and that tolerance is induced. However, CTL responses to tumor epitopes in both normal donors and cancer patient has been detected, which may indicate that tolerance is incomplete (*see, e.g.*, Kawashima *et al.*, Hum. Immunol. 59:1, 1998; Tsang, *J. Natl. Cancer Inst.* 87:82-90, 1995; Rongcun *et al.*, *J. Immunol.* 163:1037, 1999). Thus, immune tolerance does not completely eliminate or inactivate CTL precursors capable of recognizing high affinity HLA class I binding peptides.

An additional strategy to overcome tolerance is to use analog peptides. Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-

reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of peptides used in the analysis, the incidence of cross-reactivity increased from 22% to 37% (*see, e.g.*, Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II

epitopes only, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, *e.g.*, a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine can be substituted out in favor of α-amino butyric acid ("B" in the single letter abbreviations for peptide sequences listed herein). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α-amino butyric acid for cysteine not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The information in the "Fixed Nomenclature" column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, *e.g.*, a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target TAA molecules include, without limitation, CEA, MAGE, p53 and HER2/neu.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (*see, e.g.*, Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \ldots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al., J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs

(*see, e.g.*, Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia *et al*, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998; Parker *et al.*, *J. Immunol.* 152:163, 1993; Meister *et al.*, *Vaccine* 13:581, 1995; Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994; Sturniolo *et al.*, *Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC_{50} less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS' program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (*e.g.*, without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, MAGE2/3 peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

Desirably, the peptide epitope will be as small as possible while still maintaining substantially all of the immunologic activity of the native protein. When possible, it may be desirable to optimize HLA class I binding peptide epitopes of the invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptide epitopes may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules.

The identification and preparation of peptides of other lengths can also be carried out using the techniques described herein. Moreover, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (*See*, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated

under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al., J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/supermotifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the

inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon-γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp.*

Med. 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.* Alexander *et al.*, Immunity 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Exemplary immunogenic peptide epitopes are set out in Table XXIII.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses

HLA class I and class II binding peptides as described herein can be used, in one embodiment of the invention, as reagents to evaluate an immune response. The immune response to be evaluated may be induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that may be used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, a peptide of the invention may be used in a tetramer staining assay to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood

mononuclear cells. A tetramer reagent using a peptide of the invention may be generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2-microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes.

Peptides of the invention may also be used as reagents to evaluate immune recall responses (*see, e.g.*, Bertoni *et al.*, *J. Clin. Invest.* 100:503-513, 1997 and Penna *et al.*, *J. Exp. Med.* 174:1565-1570, 1991). For example, patient PBMC samples from individuals with cancer may be analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed, for example, for CTL or for HTL activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention may also be used to make antibodies, using techniques well known in the art (see, *e.g.* *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual,* Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

Vaccines that contain an immunogenically effective amount of one or more peptides as described herein are a further embodiment of the invention. Once

appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*,Vitiello, A. *et al., J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al., Molec. Immunol.* 28:287-294, 1991: Alonso *et al., Vaccine* 12:299-306, 1994; Jones *et al., Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al., Nature* 344:873-875, 1990; Hu *et al., Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al., Nature* 320:535, 1986; Hu, S. L. *et al., Nature* 320:537, 1986; Kieny, M.-P. *et al., AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al., J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al., Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al., J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. *et al., Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al., Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al., Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al., J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al., Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al., Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s). The peptide(s) can be individually linked to its own carrier; alternatively, the peptide(s) can exist as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune

response. The composition may be a naturally occurring region of an antigen or may be prepared, *e.g.*, recombinantly or by chemical synthesis.

Furthermore, useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS).

As disclosed in greater detail herein, upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142). Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality.

For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host

bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular antigen (infectious or tumor-associated antigen) are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, *e.g.*, with a minigene construct in accordance with the invention, in order to elicit immune responses. Minigenes will be discussed in greater detail in a following section.

Vaccine compositions may also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see*, *e.g.*, U.S. Patent No. 5,922,687).

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting

discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. Exemplary epitopes that may be utilized in a vaccine to treat or prevent cancer are set out in Tables XXXVII and XXXVIII. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15. The MAGE2/3 epitopes selected for inclusion are preferably conserved between the two proteins.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of particular relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the

carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, it is important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in the following section, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Furthermore, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis, that only exists because two discrete peptide sequences are encoded directly next to each other. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

IV.K.1. Minigene Vaccines

A growing body of experimental evidence demonstrates that a number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing MAGE2/3 epitopes derived from multiple regions of the MAGE2/3 proteins, the PADRE™ universal helper T cell epitope (or multiple HTL epitopes from MAGE2/3), and an endoplasmic reticulum-

translocating signal sequence can be engineered. A vaccine may also comprise epitopes, in addition to MAGE2/3 epitopes, that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested. Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.,* the human cytomegalovirus

(hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor

according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffered saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, *e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al., Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA).

Twenty-one days after immunization, splenocytes are harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in the co-pending applications U.S.S.N. 08/820,360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide may be linked to the T helper peptide without a spacer.

The CTL peptide epitope may be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated. The HTL peptide epitopes used in the invention can be modified in the same

manner as CTL peptides. For instance, they may be modified to include D-amino acids or be conjugated to other molecules such as lipids, proteins, sugars and the like.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferrably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. Specifically, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε-and α-

amino groups of a lysine residue and then linked, *e.g.,* via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.,* incomplete Freund's adjuvant. A particularly effective immunogen comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, *e.g.,* Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide (*see, e.g.*, Deres, *et al., Nature* 342:561, 1989). Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P_3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

As noted herein, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH_2 acylation, *e.g.*, by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.,* ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly humans, to treat and/or prevent cancer. Vaccine compositions containing the peptides of the invention are administered to a cancer patient or to an individual susceptible to, or

otherwise at risk for, cancer to elicit an immune response against TAAs and thus enhance the patient's own immune response capabilities. In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the tumor antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.,* the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

The vaccine compositions of the invention may also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 μg to about 50,000 μg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.,* DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

When the peptide is contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing antigen-presenting cells *in vitro* with the peptide. Such a cell population is subsequently administered to a patient in a therapeutically effective dose.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already diagnosed with

cancer. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences.

For therapeutic use, administration should generally begin at the first diagnosis of cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (i.e., including, but not limited to embodiments such as peptide cocktails, polyepitopic polypeptides, minigenes, or TAA-specific CTLs) delivered to the patient may vary according to the stage of the disease. For example, a vaccine comprising TAA-specific CTLs may be more efficacious in killing tumor cells in patients with advanced disease than alternative embodiments.

The vaccine compositions of the invention may also be used therapeutically in combination with treatments such as surgery. An example is a situation in which a patient has undergone surgery to remove a primary tumor and the vaccine is then used to slow or prevent recurrence and/or metastasis.

Where susceptible individuals, *e.g.*, individuals who may be diagnosed as being genetically pre-disposed to developing a particular type of tumor, are identified prior to diagnosis of cancer, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. The peptides and compositions of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

Thus, for treatment of cancer, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher

value is about 10,000; 20,000; 30,000; or 50,000 μg, preferably from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at established intervals, *e.g.,* from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. Administration should continue until at least clinical symptoms or laboratory tests indicate that the tumor has been eliminated or that the tumor cell burden has been substantially reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.,* intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.,* water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.,* from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see*, *e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are

the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1. HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.221-transfectants were used as sources of HLA class I molecules. These cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-

glutamine (GIBCO, Grand Island, NY), 50μM 2-ME, 100μg/ml of streptomycin, 100U/ml of penicillin (Irvine Scientific) and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells were grown in 225-cm^2 tissue culture flasks or, for large-scale cultures, in roller bottle apparatuses. The specific cell lines routinely used for purification of MHC class I and class II molecules are listed in Table XXIV.

Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al., J. Immunol.* 154:247 (1995); Sette, *et al., Mol. Immunol.* 31:813 (1994)). Briefly, cells were lysed at a concentration of 10^8 cells/ml in 50 mM Tris-HCl, pH 8.5, containing 1% Nonidet P-40 (Fluka Biochemika, Buchs, Switzerland), 150 mM NaCl, 5 mM EDTA, and 2 mM PMSF. Lysates were cleared of debris and nuclei by centrifugation at 15,000 x g for 30min.

HLA molecules were purified from lysates by affinity chromatography. Lysates prepared as above were passed twice through two pre-columns of inactivated Sepharose CL4-B and protein A-Sepharose. Next, the lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10-column volumes of 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, 2-column volumes of PBS, and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, MHC molecules were eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM ^{125}I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. The final concentrations of protease inhibitors (each from CalBioChem, La Jolla, CA) were 1 mM

PMSF, 1.3 nM 1.10 phenanthroline, 73 μM pepstatin A, 8mM EDTA, 6mM N-ethylmaleimide (for Class II assays), and 200 μM N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK). All assays were performed at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and DRB1*1601 (DR2w21β_1) and DRB4*0101 (DRw53), which were performed at pH 5.0. pH was adjusted as described elsewhere (see Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998).

Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215, Montgomeryville, PA), eluted at 1.2 mls/min with PBS pH 6.5 containing 0.5% NP40 and 0.1% NaN_3. Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2β_1) assay makes separation of bound from unbound peaks more difficult under these conditions, all DRB1*1501 (DR2w2β_1) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

Radiolabeled peptides were iodinated using the chloramine-T method. Representative radiolabeled probe peptides utilized in each assay, and its assay specific IC_{50} nM, are summarized in Tables IV and V. Typically, in preliminary experiments, each MHC preparation was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

Since under these conditions [label]<[HLA] and $IC_{50} \geq$[HLA], the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the

positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

Because the antibody used for HLA-DR purification (LB3.1) is α-chain specific, β_1 molecules are not separated from β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2β_1), DRB5*0101 (DR2w2β_2), DRB1*1601 (DR2w21β_1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DRβ molecule specificity have been described previously (*see, e.g.*, Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).

Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorthims for identification of supermotif and/or motif-bearing epitopes

The searches performed to identify the motif-bearing peptide sequences in Examples 2 and 5 employed protein sequence data for the tumor-associated antigens MAGE2/3.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated protein sequences were analyzed using a text string search software program, *e.g.*, MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$\text{“}\Delta G\text{”} = a_{1i} \times a_{2i} \times a_{3i} \ldots\ldots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (*see* also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i. For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

The complete protein sequences from MAGE2/3 were scanned, utilizing motif identification software, to identify 8-, 9-, 10-, and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

A total of 285 HLA-A2 supermotif-positive sequences were identified within the MAGE2 and/or MAGE3 protein sequences. Of these, 137 of the corresponding peptides were synthesized and tested for the capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Nineteen of the peptides bound A*0201 with IC_{50} values ≤500 nM.

The 19 A*0201-binding peptides were subsequently tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, 17 of the 19 peptides were found to be A2-supertype cross-reactive binders, binding at least three of the five A2-supertype alleles tested. One of the peptides was selected for further evaluation.

Selection of HLA-A3 supermotif-bearing epitopes

The protein sequences scanned above are also examined for the presence of peptides with the HLA-A3-supermotif primary anchors using methodology similar to that performed to identify HLA-A2 supermotif-bearing epitopes.

Peptides corresponding to the supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the two most prevalent A3-supertype alleles. The peptides that are found to bind one of the two alleles with binding affinities of ≤500 nM are then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801) to identify those that can bind at least three of the five HLA-A3-supertype molecules tested.

Selection of HLA-B7 supermotif bearing epitopes

The same target antigen protein sequences are also analyzed to identify HLA-B7-supermotif-bearing sequences. The corresponding peptides are then synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Those peptides that bind B*0702 with IC_{50} of ≤500 nM are then tested for binding to other common B7-supertype molecules (B*3501, B*5101,

B*5301, and B*5401) to identify those peptides that are capable of binding to three or more of the five B7-supertype alleles tested.

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into potential vaccine constructs. An analysis of the protein sequence data from the target antigens utilized above can also be performed to identify HLA-A1- and A24-motif-containing conserved sequences.

Example 3. Confirmation of Immunogenicity

Motif analysis and binding studies described in Example 2 identified seventeen potential epitopes for both MAGE2 and MAGE3. Four of the peptide are, however, identical in both MAGE2 and 3, and therefore do not represent distinct epitopes. A total of 13 peptides were selected for *in vitro* immunogenicity testing. Testing was performed using the following methodology:

Target Cell Lines for Cellular Screening:

The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, was used as the peptide-loaded target to measure activity of HLA-A2.1-restricted CTL. The HLA-typed melanoma cell lines (624mel and 888mel) were obtained from Y. Kawakami and S. Rosenberg, National Cancer Institute, Bethesda, MD. The cell lines were maintained in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. The melanoma cells were treated with 100U/ml IFNγ (Genzyme) for 48 hours at 37°C before use as targets in the ^{51}Cr release and *in situ* IFNγ assays.

Primary CTL Induction Cultures:

Generation of Dendritic Cells (DC): PBMCs were thawed in RPMI with 30 μg/ml DNAse, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/strpetomycin). The monocytes were purified by plating 10 x 10^6 PBMC/well in a 6-well plate. After 2 hours at 37°C, the non-adherent cells were removed by gently

shaking the plates and aspirating the supernatants. The wells were washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 were then added to each well. DC were used for CTL induction cultures following 7 days of culture.

Induction of CTL with DC and Peptide: CD8+ T-cells were isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detacha-bead® reagent. Typically about 200-250x10^6 PBMC were processed to obtain 24x10^6 $CD8^+$ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs were thawed in RPMI with 30µg/ml DNAse, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20x10^6cells/ml. The magnetic beads were washed 3 times with PBS/AB serum, added to the cells (140µl beads/20x10^6 cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells were washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at 100x10^6 cells/ml (based on the original cell number) in PBS/AB serum containing 100µl/ml detacha-bead® reagent and 30µg/ml DNAse. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads were washed again with PBS/AB/DNAse to collect the CD8+ T-cells. The DC were collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with 40µg/ml of peptide at a cell concentration of 1-2x10^6/ml in the presence of 3µg/ml $ß_2$- microglobulin for 4 hours at 20°C. The DC were then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (@1x10^5 cells/ml) were co-cultured with 0.25ml of CD8+ T-cells (@2x10^6 cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. rHuman IL10 was added the next day at a final concentration of 10 ng/ml and rhuman IL2 was added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction the cells were restimulated with peptide-pulsed adherent cells. The PBMCS were thawed and washed twice with RPMI and DNAse. The cells were resuspended at 5x10^6 cells/ml and irradiated at ~4200 rads. The PBMCs were plated at 2x10^6 in 0.5ml complete medium per well and incubated for 2 hours at 37°C. The plates were washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10µg/ml of peptide in the presence of 3 µg/ml $ß_2$ microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at

37°C. Peptide solution from each well was aspirated and the wells were washed once with RPMI. Most of the media was aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells were then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 was added at a final concentration of 10ng/ml and rhuman IL2 was added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later the cultures were assayed for CTL activity in a ^{51}Cr release assay. In some experiments the cultures were assayed for peptide-specific recognition in the in situ IFNγ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity was measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ^{51}Cr release.

Seven days after the second restimulation, cytotoxicity was determined in a standard (5hr) ^{51}Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets were prepared by incubating the cells with 10μg/ml peptide overnight at 37°C.

Adherent target cells were removed from culture flasks with trypsin-EDTA. Target cells were labelled with 200μCi of ^{51}Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10^6 per ml and diluted 1:10 with K562 cells at a concentration of $3.3x10^6$/ml (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 μl) and 100μl of effectors were plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 μl of supernatant were collected from each well and percent lysis was determined according to the formula: [(cpm of the test sample- cpm of the spontaneous ^{51}Cr release sample)/(cpm of the maximal ^{51}Cr release sample- cpm of the spontaneous ^{51}Cr release sample)] x 100. Maximum and spontaneous release were determined by incubating the labelled targets with 1% Trition X-100 and media alone, respectively. A positive culture was defined as one in which the specific lysis (sample-background) was 10% or higher in the case of individual wells and was 15% or more at the 2 highest E:T ratios when expanded cultures were assayed.

***In situ* Measurement of Human γIFN Production as an Indicator of Peptide-specific and Endogenous Recognition**

Immulon 2 plates were coated with mouse anti-human IFNγ monoclonal antibody (4 μg/ml 0.1M $NaHCO_3$, pH8.2) overnight at 4°C. The plates were washed with Ca^{2+}, Mg^{2+}-free PBS/0.05% Tween 20 and blocked with PBS/10% FCS for 2 hours, after which the CTLs (100 μl/well) and targets (100 μl/well) were added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, were used at a concentration of $1x10^6$ cells/ml. The plates were incubated for 48 hours at 37°C with 5% CO_2.

Recombinant human IFNγ was added to the standard wells starting at 400 pg or 1200pg/100μl/well and the plate incubated for 2 hours at 37°C. The plates were washed and 100 μl of biotinylated mouse anti-human IFNγ monoclonal antibody (4μg/ml in PBS/3%FCS/0.05% Tween 20) were added and incubated for 2 hours at room temperature. After washing again, 100 μl HRP-streptavidin were added and incubated for 1 hour at room temperature. The plates were then washed 6x with wash buffer, 100μl/well developing solution (TMB 1:1) were added, and the plates allowed to develop for 5-15 minutes. The reaction was stopped with 50 μl/well 1M H_3PO_4 and read at OD450. A culture was considered positive if it measured at least 50 pg of IFNγ/well above background and was twice the background level of expression.

CTL Expansion. Those cultures that demonstrated specific lytic activity against peptide-pulsed targets and/or tumor targets were expanded over a two week period with anti-CD3. Briefly, $5x10^4$ CD8+ cells were added to a T25 flask containing the following: $1x10^6$ irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, $2x10^5$ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25μM 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. rHuman IL2 was added 24 hours later at a final concentration of 200IU/ml and every 3 days thereafter with fresh media at 50IU/ml. The cells were split if the cell concentration exceeded $1x10^6$/ml and the cultures were assayed between days 13 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ^{51}Cr release assay or at $1x10^6$/ml in the *in situ* IFNγ assay using the same targets as before the expansion.

Immunogenicity of A2 supermotif-bearing peptides

The A2-supermotif cross-reactive binding peptides that were selected for further evaluation were tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide was considered to be an epitope if it induced peptide-specific CTLs in at least 2 donors (unless otherwise noted) and if those CTLs also recognized the endogenously expressed peptide.

A total of 13 peptides were screened in the cellular assay and 9 peptides were shown to induce a response in PBMCs from at least 2 normal donors. CTLs to 5 of these peptides were also able to recognize endogenously expressed peptide (Table XXVII). Two of these peptide sequences, MAGE3.159 and MAGE3.160, overlap and, while both bind to 5 allele-specific HLA molecules, MAGE3.160 binds with a higher affinity to 4 of the 5 alleles. A IFNγ *in situ* ELISA of individual CTL cultures induced with MAGE3.159 showed that cells from five wells recognized the peptide-pulsed targets, and 2 of these wells also recognized the appropriate tumor target. Additionally, MAGE3.160 induced a peptide-specific CTL response in 14 of 48 wells and 3 of these wells demonstrated endogenous recognition in the IFNγ assay.

MAGE3.112, MAGE2.157, and MAGE3.271 have also been identified as epitopes (*see, e.g.*, Kawashima *et al.*, *Human Immunol.* 59:1-14, 1998; Visseren, *Int. J. Cancer* 73:125, 1997).

*Evaluation of A*03/A11 immunogenicity*

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides.

Evaluation of B7 immunogenicity

Immunogenicity screening of the B7-supertype cross-reactive binding peptides identified in Example 2 are evaluated in a manner analogous to the evaluation of A2-and A3-supermotif-bearing peptides.

Example 4. Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or "fixed" to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoguing at Primary Anchor Residues

Peptide engineering strategies were implemented to further increase the cross-reactivity of the epitopes identified above. On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

Peptides that exhibit at least weak A*0201 binding (IC_{50} of 5000 nM or less), and carrying suboptimal anchor residues at either position 2, the C-terminal position, or both, can be fixed by introducing canonical substitutions (L at position 2 and V at the C-terminus). Those analogued peptides that show at least a three-fold increase in A*0201 binding and bind with an IC_{50} of 500 nM, or less were then tested for A2 cross-reactive binding along with their wild-type (WT) counterparts. Analogued peptides that bind at least three of the five A2 supertype alleles were then selected for cellular screening analysis.

Additionally, the selection of analogs for cellular screening analysis was further restricted by the capacity of the WT parent peptide to bind at least weakly, *i.e.*, bind at an IC_{50} of 5000nM or less, to three of more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analogued peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the WT epitope (*see, e.g.*, Parkhurst *et al., J. Immunol.* 157:2539, 1996; and Pogue *et al., Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

In the cellular screening of these peptide analogs, it is important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, tumor targets that endogenously express the epitope.

Of the 19 MAGE2/3-derived A*0201 binding peptides, 14 carried suboptimal anchor residues. Five analogs of two peptide epitopes were synthesized and tested for binding to HLA-A2 supertype molecules. MAGE3.112 analogs exhibited increased A*0201 binding affinity, but the parent peptide bound all 5 A2 supertype HLA molecules and significant improvement was not achieved. The MAGE3.220 analog, however, did demonstrate a 3-fold increase in A*0201 binding affinity and improved cross-reactive binding (Table XXII).

In addition, 24 of the 26 weak A*0201 binding peptides also met the criteria for analoguing and can be similarly analyzed for improved binding properties.

Those MAGE2/3 analogs that show improved binding relative to the wildtype peptide are evaluated in the cellular screening analysis as described in Example 3.

Using methodology similar to that used to develop HLA-A2 analogs, analogs of HLA-A3 and HLA-B7 supermotif-bearing epitopes may also be generated. For example, peptides binding at least weakly to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2. The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then tested for A3-supertype cross-reactivity. B7 supermotif-bearing peptides may, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996) and tested for binding to B7 supertype alleles.

Analoguing at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. For example, the binding capacity of a B7 supermotif-bearing peptide representing a discreet single amino acid substitution at position 1 can be analyzed. A peptide can, for example, be analogued to substitute L with F at position 1 and subsequently be evaluated for increased binding affinity/ and or increased cross-reactivity. This procedure will identify analogued peptides with modulated binding affinity.

Engineered analogs with sufficiently improved binding capacity or cross-reactivity are tested for immunogenicity as above.

Other analoguing strategies

Another form of peptide analoguing, unrelated to the anchor positions, involves the substitution of a cysteine with α-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Subtitution of α-amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and crossbinding capabilities in some instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

Example 5. Identification of peptide epitope sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

Selection of HLA-DR-supermotif-bearing epitopes

To identify HLA class II HTL epitopes, the MAGE2/3 protein sequences were analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences were selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, *e.g.*, Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select

peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The MAGE2/3-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules with an IC_{50} value of 1000 nM or less, were then tested for binding to DR5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Peptides were considered to be cross-reactive DR supertype binders if they bound at an IC_{50} value of 1000 nM or less to at least 5 of the 8 alleles tested.

Following the strategy outlined above, 97 DR supermotif-bearing sequences were identified within the MAGE2/3 protein sequences. Of those, 23 scored positive in 2 of the 3 combined DR 147 algorithms. These peptides were synthesized and tested for binding to HLA-DRB1*0101, DRB1*0401, DRB1*0701 with 13, 3, and 7 peptides binding ≤1000 nM, respectively. Of the 23 peptides tested for binding to these primary HLA molecules, 7 bound at least 2 of the 3 alleles (Table XXVIII).

These 7 peptides were then tested for binding to secondary DR supertype alleles: DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Three of the peptides bound at least 5 of the 8 alleles tested, and occurred in distinct, non-overlapping regions (Table XXIX).

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al., J. Immunol.* 149:2634-2640, 1992; Geluk *et al., J. Immunol.* 152:5742-5748, 1994; Southwood *et al., J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles. For maximum efficiency in developing vaccine candidates it would be desirable for DR3 motifs to be clustered in proximity with DR supermotif regions. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the distinct binding specificity of the

DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, the MAGE2/3 protein sequences were analyzed for conserved sequences carrying one of the two DR3 specific binding motifs (Table III) reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Twenty-three motif-positive peptides were identified. The corresponding peptides were then synthesized and tested for the ability to bind DR3 with an affinity of ≤1000 nM. Two peptides were identified that met this binding criterion (Table XXX), and thereby qualify as HLA class II high affinity binders.

The 2 DR3 binding peptides were then tested for binding to the DR supertype alleles (Table XXXI). Both DR3 binding peptides bound DRB1*1302 with an IC_{50} of 269 nM, but neither was a DR supertype cross-reactive binder. Conversely, the DR supertype cross-reactive binding peptides were also tested for DR3 binding capacity, with no measurable DR3 binding observed.

In summary, 3 DR supertype cross-reactive binding peptides were identified from the MAGE2/3 protein sequences.

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides may be analogued to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue may improve DR 3 binding.

Example 6. Immunogenicity of HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology in Example 5. Immunogenicity of HTL epitopes are evaluated in a manner analogous to the determination of immunogenicity of CTL epitopes by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from cancer patient PBMCs.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae gf=1-(SQRT(1-af)) (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula [af=1-(1-Cgf)^2].

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, total=A+B*(1-A)). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An

analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Example 8. Recognition Of Generation Of Endogenous Processed Antigens After Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens, using a transgenic mouse model.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes (as described, e.g., in Wentworth et al., *Mol. Immunol.* 32:603, 1995), for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ^{51}Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ^{51}Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with TAA expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized antigen. The choice of transgenic mouse model to be used for such an analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

Example 9. Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of a tumor associated antigen CTL/HTL peptide conjugate whereby the vaccine composition comprises peptides to be administered to a cancer patient. The peptide composition can comprise multiple CTL and/or HTL epitopes and further, can comprise epitopes selected from multiple-tumor associated antigens. The epitopes are identified using methodology as described in Examples 1-6 This analysis demonstrates the enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes

in a vaccine composition. Such a peptide composition can comprise an HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Tables XXII, XXVI, XXVII, or other analogs of that epitope. The HTL epitope is, for example, selected from Table XXXI. The peptides may be lipidated, if desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

The target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al., J. Exp. Med.* 173:1007, 1991).

In vitro CTL activation: One week after priming, spleen cells ($30x10^6$ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts ($10x10^6$ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to $1.5x10^6$) are incubated at 37°C in the presence of 200 µl of ^{51}Cr. After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10^4 ^{51}Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/10^6 cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ^{51}Cr release assay. To obtain specific lytic units/10^6, the lytic units/10^6 obtained in the absence of peptide is subtracted from the lytic units/10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the effector (E): target (T) ratio

of 50:1 (i.e., $5x10^5$ effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., $5x10^4$ effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000)-(1/500,000)] \times 10^6 = 18$ LU.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation. The magnitude and frequency of response can also be compared to the CTL response achieved using the CTL epitopes by themselves. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 10. Selection of CTL and HTL epitopes for inclusion in a cancer vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition may be in the form of a nucleic acid sequence, either single or one or more sequences (*i.e.*, minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles are balanced in order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15. The MAGE2/3 epitopes selected for inclusion are preferably conserved between the two proteins.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art and discussed herein, can be employed to assess breadth, or redundancy, of population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, a sequence that has the greatest number of epitopes per provided sequence is provided. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, the sequence is screened in order to insure that it does not have pathological or other deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in Example 11, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope, which is not present in a native protein sequence. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXII, XXVI, XXVII, and XXXI. A vaccine composition

comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response that results in tumor cell killing and reduction of tumor size or mass.

Example 11. Construction of Minigene Multi-Epitope DNA Plasmids

This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. Preferred epitopes are identified, for example, in Tables XXII, XXVI, XXVII, and XXXI. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple TAAs are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple tumor antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in

three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated Tm of each primer pair) for 30 sec, and 72°C for 1 min.

For the first PCR reaction, 5 μg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 μl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-chloride, pH 8.75, 2 mM $MgSO_4$, 0.1% Triton X-100, 100 μg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.,* in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994.

Alternatively, plasmid constructs can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines "antigenicity" and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (*see, e.g.*, Sijts *et al.*, *J. Immunol.* 156:683-692, 1996; Demotz *et al.*, *Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by infected or transfected target cells, and then determining the concentration of peptide necessary to obtained equivalent levels of lysis or lymphokine release (*see, e.g.*, Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

To assess the capacity of the minigene construct (*e.g.*, a pMin minigene construct generated as decribed in U.S.S.N. 09/311,784) to induce CTLs *in vivo*, HLA-A11/K^b transgenic mice, for example, are immunized intramuscularly with 100 μg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ^{51}Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 μg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant. CD4+ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ^3H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. Immunity 1:751-761, 1994). The results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in Example 11, may also be evaluated as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent may consist of recombinant protein (*e.g.*, Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for example, expressing a minigene or DNA encoding the complete protein of interest (*see, e.g.*, Hanke *et al.*, *Vaccine* 16:439-445, 1998; Sedegah *et al.*, *Proc. Natl. Acad. Sci USA*

95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al.*, *Nature Med.* 5:526-34, 1999).

For example, the efficacy of the DNA minigene may be evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 μg of the DNA minigene encoding the immunogenic peptides. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10^7 pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 μg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an IFN-γ ELISA. It is found that the minigene utilized in a prime-boost mode elicits greater immune responses toward the HLA-A2 supermotif peptides than with DNA alone. Such an analysis is also performed using other HLA-A11 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

Example 13. Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent cancer in persons who are at risk for developing a tumor. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to an individual at risk for a cancer, *e.g.*, melanoma. The composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freunds Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 μg, generally 100-5,000 μg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against cancer.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14. Polyepitopic Vaccine Compositions Derived from Native TAA Sequences

A native TAA polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify "relatively short" regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The "relatively short" peptide is generally less than 1,000, 500, or 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from TAAs. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native TAAs thus avoiding the need to

evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

Example 15. Polyepitopic Vaccine Compositions Directed To Multiple Tumors

The MAGE2/3 peptide epitopes of the present invention are used in conjunction with peptide epitopes from other target tumor antigens to create a vaccine composition that is useful for the treatment of various types of tumors. For example, a set of TAA epitopes can be selected that allows the targeting of most common epithelial tumors (*see, e.g.*, Kawashima *et al.*, *Hum. Immunol.* 59:1-14, 1998). Such a composition includes epitopes from CEA, HER-2/neu, and MAGE2/3, all of which are expressed to appreciable degrees (20-60%) in frequently found tumors such as lung, breast, and gastrointestinal tumors.

The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various TAAs, or can be administered as a composition comprising one or more discrete epitopes. Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

Targeting multiple tumor antigens is also important to provide coverage of a large fraction of tumors of any particular type. A single TAA is rarely expressed in the majority of tumors of a given type. For example, approximately 50% of breast tumors express CEA, 20% express MAGE3, and 30% express HER-2/neu. Thus, the use of a single antigen for immunotherapy would offer only limited patient coverage. The combination of the three TAAs, however, would address approximately 70% of breast tumors. A vaccine composition comprising epitopes from multiple tumor antigens also reduces the potential for escape mutants due to loss of expression of an individual tumor antigen.

Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a TAA. Such an analysis may be performed using multimeric complexes as described, *e.g.*, by Ogg *et al.*, *Science*

279:2103-2106, 1998 and Greten *et al.*, *Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, tumor-associated antigen HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using a TAA peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al., N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μl of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the TAA epitope, and thus the stage of tumor progression or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17. Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who are in remission, have a tumor, or who have been vaccinated with a TAA vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any TAA vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 μg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 μg/ml to each well and HBV core 128-140 epitope is added at 1 μg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4 x 10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 μl/well of complete RPMI. On days 3 and 10, 100 μl of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al., Nature Med.* 2:1104,1108, 1996; Rehermann *et al., J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al. J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al. J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 μM, and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4 hour, split-well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: 100 x [(experimental release-spontaneous release)/maximum release-spontaneous release)]. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to the TAA or TAA vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of $1.5x10^5$ cells/well and are stimulated with 10 μg/ml synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^3H-thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H-thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H-thymidine incorporation in the presence of antigen divided by the ^3H-thymidine incorporation in the absence of antigen.

Example 18. Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study. Such a trial is designed, for example, as follows:

A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage. Additional booster inoculations can be administered on the same schedule.

The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

Example 19. Therapeutic Use in Cancer Patients

Evaluation of vaccine compositions are performed to validate the efficacy of the CTL-HTL peptide compositions in cancer patients. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in cancer patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of cancer patients, as manifested by a reduction in tumor cell numbers. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females (unless the tumor is sex-specific, *e.g.*, breast or prostate cancer), and represent diverse ethnic backgrounds.

Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to evaluate the efficacy of a DNA vaccine in transgenic mice, which was described in Example 12, may also be used for the administration of the vaccine to humans. Such a vaccine regimen may include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization may be performed using an expression vector, such as that constructed in Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 μg) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of $5\text{-}10^7$ to $5\text{x}10^9$ pfu. An alternative recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polyepitopic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to achieve protective immunity against cancer is generated.

Example 21. Administration of Vaccine Compositions Using Dendritic Cells

Vaccines comprising peptide epitopes of the invention may be administered using dendritic cells. In this example, the immunogenic peptide epitopes are used to elicit a CTL and/or HTL response *ex vivo*.

Ex vivo CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptides. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Alternatively, the peptide-pulsed dendritic cells may be administered to the patient to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated as described in Example 3. The dendritic cell population is expanded and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

Example 22. Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism or transfected with nucleic acids that express the tumor antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, e.g., by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells

may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**T***L,V,M,S*		**FWY**
A2	**LIVM***A,T,Q*		**IV***M,A,T,L*
A3	**VSMA***T,L,I*		**RK**
A24	**YF***W,I,V,L,M,T*		**FI***Y,W,L,M*
B7	**P**		**VILF***M,W,Y,A*
B27	**RHK**		**FYL***W,M,I,V,A*
B44	**E***D*		**FWYLIMVA**
B58	**ATS**		**FWY***L,I,V,M,A*
B62	**QL***I,V,M,P*		**FWY***M,I,V,L,A*
MOTIFS			
A1	**TSM**		**Y**
A1		**DE***A,S*	**Y**
A2.1	**LM***V,Q,I,A,T*		**V***L,I,M,A,T*
A3	**LMVISATF***C,G,D*		**KYR***H,F,A*
A11	**VTMLISAGN***C,D,F*		**K***R,Y,H*
A24	**YFW***M*		**FLIW**
A*3101	**MVT***A,L,I,S*		**R***K*
A*3301	**MVALF***I,S,T*		**RK**
A*6801	**AVT***M,S,L,I*		**RK**
B*0702	**P**		**LMF***W,Y,A,I,V*
B*3501	**P**		**LMFWY***I,V,A*
B51	**P**		**LIVF***W,Y,A,M*
B*5301	**P**		**IMFWY***A,L,V*
B*5401	**P**		**ATIV***L,M,F,W,Y*

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE Ia

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**TI***LVMS*		**FWY**
A2	*VQAT*		**V***LIMAT*
A3	**VSMA***TLI*		**RK**
A24	**YF***WIVLMT*		**FI***YWLM*
B7	**P**		**VILF***MWYA*
B27	**RHK**		**FYL***WMIVA*
B58	**ATS**		**FWY***LIVMA*
B62	**QL***IVMP*		**FWY***MIVLA*
MOTIFS			
A1	**TSM**		**Y**
A1		**DE***AS*	**Y**
A2.1	*VQAT**		**V***LIMAT*
A3.2	**LMVISATF***CGD*		**KYR***HFA*
A11	**VTMLISAGN***CDF*		**K***RHY*
A24	**YFW**		**FLIW**

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
SUPERMOTIFS										
A1			1° Anchor TI*LVMS*							1° Anchor FWY
A2			1° Anchor LIVM*ATQ*							1° Anchor LIVMAT
A3	preferred		1° Anchor VSMA*TLI*	YFW (4/5)			YFW (3/5)	YFW (4/5)	P (4/5)	1°Anchor RK
	deleterious	DE (3/5); P (5/5)		DE (4/5)						
A24			1° Anchor YF*WIVLM* *T*							1° Anchor FI*YWLM*
B7	preferred	FWY (5/5) LIVM (3/5)	1°Anchor P	FWY (4/5)					FWY (3/5)	1°Anchor VILF*MWYA*
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN (3/5)				DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)	
B27			1° Anchor RHK							1° Anchor FYL*WMIVA*
B44			1° Anchor E*D*							1° Anchor FWYLIMVA
B58			1° Anchor ATS							1° Anchor FWY*LIVMA*
B62			1° Anchor QL*IVMP*							1° Anchor FWY*MIVLA*

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
MOTIFS										
A1 9-mer	preferred	GFYW	1°Anchor STM	DEA	YFW		P	DEQN	YFW	1°Anchor Y
	deleterious	DE		RHKLIVM P	A	G	A			
A1 9-mer	preferred	GRHK	ASTCLIV M	1°Anchor DE*AS*	GSTC		ASTC	LIVM	DE	1°Anchor Y
	deleterious	A	RHKDEPY FW		DE	PQN	RHK	PG	GP	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 10-mer	peferred	YFW	1°Anchor STM	DEAQN	A	YFWQN		PASTC	GDE	P	1°Anchor Y
	deleterious	GP		RHKGLIV M	DE	RHK	QNA	RHKYFW	RHK	A	
A1 10-mer	preferred	YFW	STCLIVM	1°Anchor DE*AS*	A	YFW		PG	G	YFW	1°Anchor Y
	deleterious	RHK	RHKDEPY FW			P	G		PRHK	QN	
A2.1 9-mer	preferred	YFW	1°Anchor LM*IVQAT*	YFW	STC	YFW		A	P	1°Anchor V*LIMAT*	
	deleterious	DEP		DERKH			RKH	DERKH			
A2.1 10-mer	preferred	AYFW	1°Anchor LM*IVQAT*	LVIM	G		G		FYWL VIM		1°Anchor V*LIMAT*
	deleterious	DEP		DE	RKHA	P		RKH	DERK H	RKH	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3	preferred	RHK	1°Anchor LMVISAT F*CGD*	YFW	PRHKYFW	A	YFW		P	1°Anchor KYR*HFA*	
	deleterious	DEP		DE							
A11	preferred	A	1°Anchor VTLMISA GN*CDF*	YFW	YFW	A	YFW	YFW	P	1°Anchor K*RYH*	
	deleterious	DEP						A	G		
A24 9-mer	preferred	YFWRHK	1°Anchor YFW*M*		STC			YFW	YFW	1°Anchor FLIW	
	deleterious	DEG		DE	G	QNP	DERHK	G	AQN		
A24 10-mer	preferred		1°Anchor YFW*M*		P	YFWP		P			1°Anchor FLIW
	deleterious			GDE	QN	RHK	DE	A	QN	DEA	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3101	preferred	RHK	1°Anchor MVT*ALIS*	YFW	P		YFW	YFW	AP	1°Anchor R*K*	
	deleterious	DEP		DE		ADE	DE	DE	DE		
A3301	preferred		1°Anchor MVALF*IS T*	YFW				AYFW		1°Anchor RK	
	deleterious	GP		DE							
A6801	preferred	YFWSTC	1°Anchor AVT*MSLI*			YFWLIV M		YFW	P	1°Anchor RK	
	deleterious	GP		DEG		RHK			A		
B0702	preferred	RHKFWY	1°Anchor P	RHK		RHK	RHK	RHK	PA	1°Anchor LMF*WYAIV*	
	deleterious	DEQNP		DEP	DE	DE	GDE	QN	DE		
B3501	preferred	FWYLIVM	1°Anchor P	FWY				FWY		1°Anchor LMFWY*IVA*	
	deleterious	AGP				G	G				

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
B51	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		G	FWY	1°Anchor LIVF*WYAM*	
	deleterious	AGPDERHKSTC				DE	G	DEQN	GDE		
B5301	preferred	LIVMFWY	1°Anchor P	FWY	STC	FWY		LIVMFWY	FWY	1°Anchor IMFWY*ALV*	
	deleterious	AGPQN					G	RHKQN	DE		
B5401	preferred	FWY	1°Anchor P	FWYLIVM		LIVM		ALIVM	FWYAP	1°Anchor ATIV*LMFW Y*	
	deleterious	GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE		

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.

TABLE III

		POSITION								
MOTIFS		1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	FMY*LIVW*	M	T		I	VST*CPALIM*	MH		MH
	deleterious				W			R		WDE
DR1	preferred	MF*LIVWY*			PAMQ		VMAT*SPLIC*	M		AVM
	deleterious		C	CH	FD	CWD		GDE	D	
DR7	preferred	MF*LIVWY*	M	W	A		IVMSA*CTPL*	M		IV
	deleterious		C		G			GRD	N	G
DR Supermotif		MF*LIVWY*					VMSTA*CPLI*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

Table IV. HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVYLL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2β1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2β2	553.01	QYIKANSKFIGITE	20

The "Nomenclature" column lists the allelic designations used in Tables XIX and XX.

Table VI

HLA-supertype	Allelle-specific HLA-supertype members	
	Verified[a]	Predicted[b]
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

a. Verified alleles inclueds alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VII A

Mage 2 A01 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASEYLQLVF	154	9		1
ASSFSTTINY	68	10	0 1700	2
DLVQENYLEY	249	10		3
ELSMLEVF	224	8		4
ELVHFLLLKY	115	10		5
ESVLRNCQDF	137	10		6
ESVLRNCQDFF	137	11		7
EVFEGREDSVF	229	11		8
EVVPISHLY	168	9	0.0028	9
FSTTINYTLW	71	10		10
GSDPACYEF	263	9		11
GSDPACYEFLW	263	11		12
HSPQGASSF	63	9		13
ILVTCLGLSY	177	10		14
ISRKMVELVHF	109	11		15
KIGGEPHSY	292	10		16
KMVELVHF	112	8		17
LLMQDLVQENY	245	11		18
LMQDLVQENY	246	10	0 0450	19
LVHFLLLKY	116	9		20
LVQENYLEY	250	9		21
LVTCLGLSY	178	9		22
PVIFSKASEY	148	10		23
QVPGSDPACY	260	10		24
RMFPDLESEF	96	10		25
SSFSTTINY	69	9	0.0430	26
STTINYTLW	72	9		27
SVLRNCQDF	138	9		28
SVLRNCQDFF	138	10		29
TTINYTLW	73	8		30
VIFSKASEY	149	9		31
VLRNCQDF	139	8		32
VLRNCQDFF	139	9		33
VTCLGLSY	179	8		34
VVEVVPISHLY	166	11	0 2000	35
VVPISHLY	169	8		36
YILVTCLGLSY	176	11		37

Table VII B

Mage 3 A01 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASSLPTTMNY	68	10	2.6000	38
ASSSLQLVF	154	9		39
ATCLGLSY	179	8	0.1100	40
ELSVLEVF	224	8		41
ELVHFLLLKY	115	10		42
EMLGSVVGNW	134	10		43
EVDPIGHLY	168	9	18.0000	44
EVDPIGHLYIF	168	11		45
FVQENYLEY	250	9		46
GSDPACYEF	263	9		47
GSDPACYEFLW	263	11		48
GSVVGNWQY	137	9	0.0500	49
GSVVGNWQYF	137	10		50
GSVVGNWQYFF	137	11		51
HISYPPLHEW	298	10		52
ISGGPHISY	293	9	0.0370	53
ISYPPLHEW	299	9		54
KISGGPHISY	292	10	0.0011	55
KVAELVHF	112	8		56
LLTQHFVQENY	245	11		57
LMEVDPIGHLY	166	11	7.5000	58
LSRKVAELVHF	109	11		59
LTQHFVQENY	246	10	0.2600	60
LVHFLLLKY	116	9		61
MLGSVVGNW	135	9		62
MLGSVVGNWQY	135	11		63
PIGHLYIF	171	8		64
PSTFPDLESEF	95	11		65
PTTMNYPLW	72	9		66
QVPGSDPACY	260	10		67
SLPTTMNY	70	8		68
SLPTTMNYPLW	70	11		69
SSLPTTMNY	69	9	0 0550	70
SSSLQLVF	155	8		71
STFPDLESEF	96	10		72
SVVGNWQY	138	8		73
SVVGNWQYF	138	9		74
SVVGNWQYFF	138	10		75
TMNYPLWSQSY	74	11	0.0830	76
TTMNYPLW	73	8		77
VVGNWQYF	139	8		78
VVGNWQYFF	139	9		79
YIFATCLGLSY	176	11		80

Table VIII A

Mage 2 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
AAISRKMV	107	8						81
AAISRKMVEL	107	10	0.0001					82
AAISRKMVELV	107	11						83
AIIAIEGDCA	207	10	0 0023					84
AISRKMVEL	108	9	0.0003					85
AISRKMVELV	108	10	0.0001					86
ALGLVGAQA	22	9	0.0030					87
ALGLVGAQAPA	22	11						88
ALIETSYV	277	8						89
ALIETSYVKV	277	10	0.0100	0 0059	0.0800	0.0019	0.0130	90
ALIETSYVKVL	277	11						91
AQAPATEEQQT	28	11						92
ATEEQQTA	32	8						93
CAPEEKIWEEL	215	11						94
CLGLSYDGL	181	9	0.0004					95
CLGLSYDGLL	181	10	0.0001					96
CQDFFPVI	143	8						97
DLESEFQA	100	8						98
DLESEFQAA	100	9	0 0001					99
DLESEFQAAI	100	10	0 0001					100
DLVQENYL	249	8						101
EALGLVGA	21	8						102
EALGLVGAQA	21	10	0.0001					103
EARGEALGL	17	9	0 0001					104
EARGEALGLV	17	10	0.0001					105
ELVHFLLL	115	8						106
EQQTASSSST	35	10						107
EQQTASSSSTL	35	11						108
ETSYVKVL	280	8						109
ETSYVKVLIIIIT	280	11						110
EVFEGREDSV	229	10	0.0003					111
EVTLGEVPA	47	9	0 0001					112
EVTLGEVPAA	47	10	0 0001					113
EVVEVVPI	165	8						114
EVVEVVPISHL	165	11						115
EVVPISHL	168	8						116
EVVPISHLYI	168	10	0 0002					117
EVVPISHLYIL	168	11						118
FAHPRKLL	239	8						119
FAHPRKLLM	239	9						120
FLLLKYRA	119	8						121
FLWGPRAL	271	8						122
FLWGPRALI	271	9	0 0470					123
FLWGPRALIET	271	11						124
FQAAISRKM	105	9						125
FQAAISRKMV	105	10						126
GASSFSTT	67	8						127
GASSFSTTI	67	9	0.0001					128
GIEVVEVV	163	8						129
GIEVVEVVPI	163	10	0.0002					130

Table VIIIA

Mage 2 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
GLEARGEA	15	8						131
GLEARGEAL	15	9	0 0001					132
GLEARGEALGL	15	11						133
GLLGDNQV	188	8						134
GLLGDNQVM	188	9	0.0038					135
GLLIIVLA	200	8						136
GLLIIVLAI	200	9	0 0002					137
GLLIIVLAII	200	10	0.0005					138
GLLIIVLAIIA	200	11						139
GLSYDGLL	183	8						140
GLVGAQAPA	24	9	0 0003					141
GLVGAQAPAT	24	10	0 0004					142
HISYPPLHERA	298	11						143
HLYILVTCL	174	9	0.0034					144
HLYILVTCLGL	174	11						145
HTLKIGGEPHI	289	11						146
IAIEGDCA	209	8						147
IIAIEGDCA	208	9	0 0001					148
IIVLAIIA	203	8						149
IIVLAIIAI	203	9	0 0009					150
ILVTCLGL	177	8						151
IVLAIIAI	204	8						152
KAEMLESV	132	8						153
KAEMLESVL	132	9	0 0001					154
KASEYLQL	153	8						155
KASEYLQLV	153	9	0.0110					156
KIGGEPHI	292	8						157
KIWEELSM	220	8						158
KIWEELSML	220	9	0.0300	0 0067	0 0570	0 1300	0.0017	159
KIWEELSMLEV	220	11	0.2800					160
KLLMQDLV	244	8						161
KMVELVHFL	112	9	0.1600					162
KMVELVHFLL	112	10	0 1100					163
KMVELVHFLLL	112	11	0 6700	0.4500	6 0000	0 6000	0 2200	164
KTGLLIIV	198	8						165
KTGLLIIVL	198	9	0 0002					166
KTGLLIIVLA	198	10	0.0002					167
KTGLLIIVLAI	198	11						168
KVLIIITLKI	285	9	0 0008					169
LAIIAIEGDCA	206	11						170
LIETSYVKV	278	9	0 0001					171
LIETSYVKVL	278	10	0 0003					172
LIIVLAII	202	8						173
LIIVLAIIA	202	9	0 0008					174
LIIVLAIIAI	202	10	0 0013					175
LLGDNQVM	189	8						176
LLGDNQVMPKT	189	11						177
LLIIVLAI	201	8						178
LLIIVLAII	201	9	0.0001					179
LLIIVLAIIA	201	10	0.0002					180

Table VIIIA

Mage 2 A02 Supermotif with Binding Data

Sequence	Position	No of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
LLIIVLAIIAI	201	11						181
LLKYRAREPV	121	10	0 0001					182
LLKYRAREPVT	121	11						183
LLLKYRAREPV	120	11	0.0001					184
LMQDLVQENYL	246	11						185
LQLVFGIEV	158	9						186
LQLVFGIEVV	158	10						187
LVEVTLGEV	45	9	0.0001					188
LVEVTLGEVPA	45	11						189
LVFGIEVV	160	8						190
LVFGIEVVEV	160	10	0 0120					191
LVFGIEVVEVV	160	11						192
LVGAQAPA	25	8						193
LVGAQAPAT	25	9	0 0001					194
LVHFLLLKYRA	116	11						195
MQDLVQENYL	247	10						196
MVELVHFL	113	8						197
MVELVHFLL	113	9	0.0031					198
MVELVHFLLL	113	10	0.0017					199
NQEEEGPRM	89	9						200
NQVMPKTGL	193	9						201
NQVMPKTGLL	193	10						202
NQVMPKTGLLI	193	11						203
PATEEQQT	31	8						204
PATEEQQTA	31	9						205
PISHLYIL	171	8						206
PISHLYILV	171	9	0 0005					207
PISHLYILVT	171	10	0 0003					208
PQGASSFST	65	9						209
PQGASSFSTT	65	10						210
PQGASSFSTTI	65	11						211
PVIFSKASEYL	148	11						212
PVTKAEML	129	8						213
PVTKAEMLESV	129	11						214
QAAISRKM	106	8						215
QAAISRKMV	106	9	0 0001					216
QAAISRKMVEL	106	11						217
QAPATEEQQT	29	10						218
QAPATEEQQTA	29	11						219
QLVFGIEV	159	8						220
QLVFGIEVV	159	9	0 0038					221
QLVFGIEVVEV	159	11	0 0018					222
QQTASSSST	36	9						223
QQTASSSSTL	36	10						224
QQTASSSSTLV	36	11						225
QTASSSST	37	8						226
QTASSSSTL	37	9	0 0002					227
QTASSSSTLV	37	10	0.0003					228
QVMPKTGL	194	8						229
QVMPKTGLL	194	9	0 0001					230

Table VIII A

Mage 2 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
QVMPKTGLLI	194	10	0.0002					231
QVMPKTGLLII	194	11						232
QVPGSDPA	260	8						233
RALIETSYV	276	9	0.0017					234
RALIETSYVKV	276	11						235
RAREPVTKA	125	9						236
RAREPVTKAEM	125	11						237
RQVPGSDPA	259	9						238
SQHCKPEEGL	7	10						239
STLVEVTL	43	8						240
STLVEVTLGEV	43	11	0.0140					241
STTINYTL	72	8						242
SVFAHPRKL	237	9	0.0046					243
SVFAHPRKLL	237	10	0.0011					244
SVFAHPRKLLM	237	11						245
TASSSSTL	38	8						246
TASSSSTLV	38	9	0.0001					247
TASSSSTLVEV	38	11						248
TLGEVPAA	49	8						249
TLKIGGEPHI	290	10	0.0001					250
TLVEVTLGEV	44	10	0.0250	0.0320	1.6000	0.0039	0.1600	251
VIFSKASEYL	149	10	0.0014					252
VLHHTLKI	286	8						253
VLRNCQDFFPV	139	11						254
VMPKTGLL	195	8						255
VMPKTGLLI	195	9	0.0010					256
VMPKTGLLII	195	10	0.0009					257
VMPKTGLLIIV	195	11						258
VQENYLEYRQV	251	11						259
VTCLGLSYDGL	179	11						260
VTKAEMLESV	130	10						261
VTKAEMLESVL	130	11						262
VTLGEVPA	48	8						263
VTLGEVPAA	48	9	0.0045					264
VVEVVPISHL	166	10	0.0002					265
VVPISHLYI	169	9	0.0002					266
VVPISHLYIL	169	10	0.0002					267
VVPISHLYILV	169	11						268
YILVTCLGL	176	9	0.0014					269
YLQLVFGI	157	8						270
YLQLVFGIEV	157	10	0.3700					271
YLQLVFGIEVV	157	11						272
YVKVLHHIT	283	8						273
YVKVLHHITL	283	9	0.0001					274
YVKVLHHITLKI	283	11						275

Table VIIIB

Mage 3 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO
AALSRKVA	107	8						276
AALSRKVAEL	107	10	0.0007					277
AALSRKVAELV	107	11						278
AASSSSTL	38	8						279
AASSSSTLV	38	9	0.0001					280
AASSSSTLVEV	38	11						281
AIIAREGDCA	207	10	0 0002					282
ALGLVGAQA	22	9	0.0030					283
ALGLVGAQAPA	22	11						284
ALSRKVAEL	108	9	0 0050					285
ALSRKVAELV	108	10	0.0001					286
ALVETSYV	277	8						287
ALVETSYVKV	277	10	0.0024					288
ALVETSYVKVL	277	11						289
AQAPATEEQEA	28	11						290
ATCLGLSYDGL	179	11						291
ATEEQEAA	32	8						292
CAPEEKIWEEL	215	11						293
CLGLSYDGL	181	9	0.0004					294
CLGLSYDGLL	181	10	0.0001					295
DLESEFQA	100	8						296
DLESEFQAA	100	9	0 0001					297
DLESEFQAAL	100	10	0.0001					298
EAASSSST	37	8						299
EAASSSSTL	37	9	0 0001					300
EAASSSSTLV	37	10	0 0001					301
EALGLVGA	21	8						302
EALGLVGAQA	21	10	0 0001					303
EARGEALGL	17	9	0 0001					304
EARGEALGLV	17	10	0 0001					305
ELMEVDPI	165	8						306
ELMEVDPIGHL	165	11	0 0260					307
ELVHFLLL	115	8						308
EQEAASSSST	35	10						309
EQEAASSSSTL	35	11						310
ETSYVKVL	280	8						311
ETSYVKVLHHM	280	11						312
EVDPIGHL	168	8						313
EVDPIGHLYI	168	10	0 0002					314
EVFEGREDSI	229	10	0 0001					315
EVFEGREDSIL	229	11						316
EVTLGEVPA	47	9	0 0001					317
EVTLGEVPAA	47	10	0 0001					318
FLLLKYRA	119	8						319
FLWGPRAL	271	8						320
FLWGPRALV	271	9	0.0820	0 0500	0.9100	0 0043	1 1000	321
FLWGPRALVET	271	11						322
FQAALSRKV	105	9						323
FQAALSRKVA	105	10						324
GASSLPTT	67	8						325

Table VIII B

Mage 3 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
GASSLPTTM	67	9	0.0001					326
GIELMEVDPI	163	10	0.0002					327
GLEARGEA	15	8						328
GLEARGEAL	15	9	0.0001					329
GLEARGEALGL	15	11						330
GLLGDNQI	188	8						331
GLLGDNQIM	188	9						332
GLLIIVLA	200	8						333
GLLIIVLAI	200	9	0 0002					334
GLLIIVLAII	200	10	0.0005					335
GLLIIVLAIIA	200	11						336
GLSYDGLL	183	8						337
GLVGAQAPA	24	9	0.0003					338
GLVGAQAPAT	24	10	0.0004					339
HISYPPLIIEWV	298	11						340
HLYIFATCL	174	9	0 0003					341
HLYIFATCLGL	174	11	0.0410	0.0140	0 1500	0 0029	0.1500	342
HMVKISGGPHI	289	11						343
IAREGDCA	209	8						344
IIAREGDCA	208	9	0.0001					345
IIVLAIIA	203	8						346
ILGDPKKL	238	8						347
ILGDPKKLL	238	9	0 0001					348
ILGDPKKLLT	238	10	0.0001					349
IMPKAGLL	195	8						350
IMPKAGLLI	195	9	0 0064					351
IMPKAGLLII	195	10	0 0015					352
IMPKAGLLIIV	195	11	0 0130					353
KAEMLGSV	132	8						354
KAEMLGSVV	132	9	0.0001					355
KAGLLIIV	198	8						356
KAGLLIIVL	198	9	0 0002					357
KAGLLIIVLA	198	10	0.0002					358
KAGLLIIVLAI	198	11						359
KASSSLQL	153	8						360
KASSSLQLV	153	9	0.0005					361
KISGGPHI	292	8						362
KIWEELSV	220	8						363
KIWEELSVL	220	9	0.0140	0 0064	0 0073	0 0590	0.0012	364
KIWEELSVLEV	220	11						365
KLLTQIIFV	244	8						366
KVAELVHFL	112	9	0.0550					367
KVAELVHFLL	112	10	0 0120					368
KVAELVHFLLL	112	11						369
KVLIIHMVKI	285	9	0 0026					370
LAIIAREGDCA	206	11						371
LIIVLAII	202	8						372
LIIVLAIIA	202	9	0 0008					373
LLGDNQIM	189	8						374
LLGDNQIMPKA	189	11						375

Table VIII B

Mage 3 A02 Supermotif with Binding Data

Sequence	Position	No of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
LLIIVLAI	201	8						376
LLIIVLAII	201	9	0 0001					377
LLIIVLAIIA	201	10	0.0002					378
LLKYRAREPV	121	10	0.0001					379
LLKYRAREPVT	121	11						380
LLLKYRAREPV	120	11	0 0001					381
LMEVDPIGHL	166	10	0.0005					382
LQLVFGIEL	158	9						383
LQLVFGIELM	158	10						384
LTQHFVQENYL	246	11						385
LVETSYVKV	278	9	0 0001					386
LVETSYVKVL	278	10	0.0002					387
LVEVTLGEV	45	9	0.0001					388
LVEVTLGEVPA	45	11						389
LVFGIELM	160	8						390
LVFGIELMEV	160	10	0 1100					391
LVGAQAPA	25	8						392
LVGAQAPAT	25	9	0 0001					393
LVHFLLLKYRA	116	11						394
MVKISGGPHI	290	10	0 0002					395
NQEEEGPST	89	9						396
NQIMPKAGL	193	9						397
NQIMPKAGLL	193	10						398
NQIMPKAGLLI	193	11						399
PATEEQEA	31	8						400
PATEEQEAA	31	9	0 0001					401
PIGHLYIFA	171	9	0 0001					402
PIGHLYIFAT	171	10	0 0003					403
PQGASSLPT	65	9						404
PQGASSLPTT	65	10						405
PQGASSLPTTM	65	11						406
PQSPQGASSL	62	10						407
PTTMNYPL	72	8						408
PVIFSKASSSL	148	11						409
PVTKAEML	129	8						410
PVTKAEMLGSV	129	11						411
QAALSRKV	106	8						412
QAALSRKVA	106	9	0.0001					413
QAALSRKVAEL	106	11						414
QAPATEEQEA	29	10	0.0001					415
QAPATEEQEAA	29	11						416
QIMPKAGL	194	8						417
QIMPKAGLL	194	9	0 0001					418
QIMPKAGLLI	194	10	0 0006					419
QIMPKAGLLII	194	11						420
QLVFGIEL	159	8						421
QLVFGIELM	159	9	0.0010					422
QLVFGIELMEV	159	11	0.3400					423
QVPGSDPA	260	8						424
RALVETSYV	276	9	0.0001					425

Table VIII B

Mage 3 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
RALVETSYVKV	276	11						426
RAREPVTKA	125	9						427
RAREPVTKAEM	125	11						428
RQVPGSDPA	259	9						429
SILGDPKKL	237	9	0.0001					430
SILGDPKKLL	237	10	0.0002					431
SILGDPKKLLT	237	11						432
SLPTTMNYPL	70	10	0.0035					433
SLQLVFGI	157	8						434
SLQLVFGIEL	157	10	0.0049					435
SLQLVFGIELM	157	11						436
SQHCKPEEGL	7	10						437
STLVEVTL	43	8						438
STLVEVTLGEV	43	11	0.0140					439
TLGEVPAA	49	8						440
TLVEVTLGEV	44	10	0.0250	0.0320	1.6000	0.0039	0.1600	441
TQHFVQENYL	247	10						442
VAELVHFL	113	8						443
VAELVHFLL	113	9	0.0001					444
VAELVHFLLL	113	10	0 0009					445
VIFSKASSSL	149	10	0.0001					446
VLHHIMVKI	286	8						447
VQENYLEYRQV	251	11						448
VTKAEMLGSV	130	10	0 0002					449
VTKAEMLGSVV	130	11						450
VTLGEVPA	48	8						451
VTLGEVPAA	48	9	0 0045					452
VVGNWQYFFPV	139	11						453
WQYFFPVI	143	8						454
YIFATCLGL	176	9	0.0180					455
YVKVLHHIM	283	8						456
YVKVLHHIMV	283	9	0 0001					457
YVKVLHHIMVKI	283	11						458

Table IX A

Mage 2 A03 Supermotif with Binding Data

Sequence	Position	No of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
AIEGDCAPEEK	210	11	0.0009	0.0007				459
ALIETSYVK	277	9	0.0810	0.1900	0.0200	0.0003	0.0280	460
DLVQENYLEYR	249	11	0.0047	0.0018				461
DSVFAHPR	236	8	-0.0004	0.0005				462
DSVFAHPRK	236	9	0.0021	0.0025	0.0006	0.0190	0.0460	463
ELSMLEVFEGR	224	11	0.0016	0.0008				464
ELVHFLLLK	115	9	0.0045	0.0011				465
ELVHFLLLKYR	115	11	0.0011	0.0031				466
EMLESVLR	134	8	-0.0009	-0.0003				467
ESEFQAAISR	102	10	0.0002	0.0002				468
ESEFQAAISRK	102	11	0.0010	0.0004				469
FLLLKYRAR	119	9						470
FSTTINYTLWR	71	11	0.0110	0.0170	0.0700	0.0074	0.0490	471
GLLGDNQVMPK	188	11	0.0780	0.0047	-0.0006	-0.0013	-0.0001	472
GSSNQEEEGPR	86	11	-0.0002	-0.0002				473
HISYPPLHER	298	10	0.0074	0.0018				474
ISYPPLHER	299	9	0.0340	0.0280	0.7700	0.8100	0.0990	475
KAEMLESVLR	132	10	0.0002	0.0009	0.0084	0.0047	0.0004	476
KVLHHTLK	285	8	0.0053	0.0100				477
LIETSYVK	278	8	-0.0004	0.0027				478
LLGDNQVMPK	189	10	0.0093	0.0014				479
LLLKYRAR	120	8	-0.0009	-0.0004				480
LSMLEVFEGR	225	10	-0.0004	0.0001				481
LVHFLLLK	116	8	0.0290	0.1500	0.0007	-0.0009	0.0200	482
LVHFLLLKYR	116	10	0.0260	0.0022				483
LVQENYLEYR	250	10	0.0027	0.0089				484
MLEVFEGR	227	8	-0.0009	-0.0004				485
MVELVHFLLLK	113	11	0.0200	0.0120	0.0038	0.0056	0.0220	486
PACYEFLWGPR	266	11	-0.0009	-0.0002				487
PLEQRSQHCK	2	10	0.0003	0.0002				488
PLHERALR	303	8	-0.0009	-0.0004				489
RALIETSYVK	276	10	0.0200	0.0750	0.0064	0.0003	0.0026	490
RAREPVTK	125	8	-0.0009	-0.0003				491
SMLEVFEGR	226	9	0.0020	0.0220	0.4900	3.2000	0.0044	492
SSNQEEEGPR	87	10	0.0002	0.0002				493
STTINYTLWR	72	10	0.0014	0.0910				494
SVFAHPRK	237	8	0.1410	0.0810	0 0130	0.0010	0.0440	495
TINYTLWR	74	8	0.0140	0.0550	0.0250	0.0370	0.3800	496
TTINYTLWR	73	9	0.0890	1.1000				497
YVKVLHHTLK	283	10	0.0033	0.0160	0.0005	-0.0009	0.0360	498

Table IX B

Mage 3 A03 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
ALVETSYVK	277	9	0.0270	0 1700	0.0009	0.0004	0.0022	499
DSILGDPK	236	8	-0.0004	-0.0003				500
DSILGDPKK	236	9	-0 0003	-0 0002				501
ELSVLEVFEGR	224	11	-0 0009	0.0023				502
ELVHFLLLK	115	9	0.0045	0 0011				503
ELVHFLLLKYR	115	11	0.0011	0 0031				504
ESEFQAALSR	102	10	0.0002	0 0002				505
ESEFQAALSRK	102	11	0.0002	0.0004				506
FLLLKYRAR	119	9						507
FVQENYLEYR	250	10	0 0009	0 0012				508
GLLGDNQIMPK	188	11	0 1300	0.0570	-0.0006	-0.0013	-0.0001	509
HVLAHAR	203	9	0.0069	0 0011				510
IVLAHAR	204	8	0.0053	0 0037				511
KVLHHMVK	285	8	0 0580	0.0190	0.0012	0.0052	-0.0001	512
LHVLAHAR	202	10	0 0280	0 0021				513
LLGDNQIMPK	189	10	0.0200	0.0110				514
LLHVLAHAR	201	11	0 0021	0 0056				515
LLLKYRAR	120	8	-0 0009	-0.0004				516
LSVLEVFEGR	225	10	-0.0006	0 0030				517
LVETSYVK	278	8	-0.0004	0 0014				518
LVHFLLLK	116	8	0 0290	0 1500	0 0007	-0 0009	0.0200	519
LVHFLLLKYR	116	10	0 0260	0.0022				520
PACYEFLWGPR	266	11	-0.0009	-0 0002				521
PLEQRSQHCK	2	10	0.0003	0.0002				522
PLHEWVLR	303	8	-0.0009	-0 0003				523
RALVETSYVK	276	10	0 0190	0 1100	0 0034	0.0003	0.0004	524
RAREPVTK	125	8	-0 0009	-0 0003				525
SILGDPKK	237	8	-0.0009	0 0012				526
SVLEVFEGR	226	9	0 0003	0 1400	0 1700	0.6600	0 0860	527
VAELVHFLLLK	113	11	-0 0002	0 0011				528
VLEVFEGR	227	8	0 0016	0 0005				529
YVKVLHHMVK	283	10	0 0020	0 0061				530

Table XA
Mage 2 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
AISRKMVEL	108	9		531
ALIETSYVKVL	277	11		532
CLGLSYDGL	181	9		533
CLGLSYDGLL	181	10		534
CYEFLWGPRAL	268	11	0.0004	535
DLESEFQAAI	100	10		536
DLVQENYL	249	8		537
DLVQENYLEY	249	10		538
EFLWGPRAL	270	9	0.0006	539
EFLWGPRALI	270	10	0.0097	540
EFQAAISRKM	104	10	0.0002	541
ELSMLEVF	224	8		542
ELVHFLLL	115	8		543
ELVHFLLLKY	115	10		544
ETSYVKVL	280	8		545
EVFEGREDSVF	229	11		546
EVVEVVPI	165	8		547
EVVEVVPISHL	165	11		548
EVVPISHL	168	8		549
EVVPISHLY	168	9		550
EVVPISHLYI	168	10		551
EVVPISHLYIL	168	11		552
EYLQLVFGI	156	9	3.5000	553
FLWGPRAL	271	8		554
FLWGPRALI	271	9		555
GIEVVEVVPI	163	10		556
GLEARGEAL	15	9		557
GLEARGEALGL	15	11		558
GLLGDNQVM	188	9		559
GLLHVLAI	200	9		560
GLLHVLAII	200	10		561
GLSYDGLL	183	8		562
HLYILVTCL	174	9		563
HLYILVTCLGL	174	11		564
HTLKIGGEPHI	289	11		565
IFSKASEY	150	8		566
IFSKASEYL	150	9	0 0230	567
IFSKASEYLQL	150	11	0 0950	568
IIVLAIIAI	203	9		569
ILVTCLGL	177	8		570
ILVTCLGLSY	177	10		571
IVLAIIAI	204	8		572
IWEELSML	221	8	0 0007	573
IWEELSMLEVF	221	11	0 0170	574
KIGGEPHI	292	8		575
KIGGEPHISY	292	10		576
KIWEELSM	220	8		577
KIWEELSML	220	9		578
KMVELVHF	112	8	0 0005	579
KMVELVHFL	112	9		580

Table X A

Mage 2 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
KMVELVHFLL	112	10		581
KMVELVHFLLL	112	11		582
KTGLLHVL	198	9		583
KTGLLHVLAI	198	11		584
KVLHHTLKI	285	9		585
LIETSYVKVL	278	10		586
LHVLAH	202	8		587
LHVLAHAI	202	10		588
LLGDNQVM	189	8		589
LLHVLAI	201	8		590
LLHVLAH	201	9		591
LLHVLAHAI	201	11		592
LLMQDLVQENY	245	11		593
LMQDLVQENY	246	10		594
LMQDLVQENYL	246	11		595
LVHFLLLKY	116	9		596
LVQENYLEY	250	9		597
LVTCLGLSY	178	9		598
LWGPRALI	272	8	0.1200	599
LYILVTCL	175	8	0.0086	600
LYILVTCLGL	175	10	0.0140	601
MFPDLESEF	97	9	0.0140	602
MVELVHFL	113	8		603
MVELVHFLL	113	9		604
MVELVHFLLL	113	10		605
PISHLYIL	171	8		606
PVIFSKASEY	148	10		607
PVIFSKASEYL	148	11		608
PVTKAEML	129	8		609
QTASSSTL	37	9		610
QVMPKTGL	194	8		611
QVMPKTGLL	194	9		612
QVMPKTGLLI	194	10		613
QVMPKTGLLH	194	11		614
QVPGSDPACY	260	10		615
RMFPDLESEF	96	10	0.0016	616
SFSTTINY	70	8		617
SFSTTINYTL	70	10	0.0150	618
SFSTTINYTLW	70	11	0.0280	619
STLVEVTL	43	8		620
STTINYTL	72	8		621
STTINYTLW	72	9		622
SVFAHPRKL	237	9		623
SVFAHPRKLL	237	10		624
SVFAHPRKLLM	237	11		625
SVLRNCQDF	138	9		626
SVLRNCQDFF	138	10		627
SYPPLHERAL	300	10	0.0003	628
SYVKVLHHTL	282	10	0.1600	629
TLKIGGEPHI	290	10		630

Table X A

Mage 2 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
TTINYTLW	73	8		631
VFAHPRKL	238	8	0.0005	632
VFAHPRKLL	238	9	0.0006	633
VFAHPRKLLM	238	10		634
VFEGREDSVF	230	10	0.0004	635
VIFSKASEY	149	9		636
VIFSKASEYL	149	10		637
VLHHTLKI	286	8		638
VLRNCQDF	139	8		639
VLRNCQDFF	139	9		640
VMPKTGLL	195	8	-0.0004	641
VMPKTGLLI	195	9	0.2300	642
VMPKTGLLII	195	10	0.0580	643
VTCLGLSY	179	8		644
VTCLGLSYDGL	179	11		645
VTKAEMLESVL	130	11		646
VVEVVPISHL	166	10		647
VVEVVPISHLY	166	11		648
VVPISHLY	169	8		649
VVPISHLYI	169	9		650
VVPISHLYIL	169	10		651
YILVTCLGL	176	9		652
YILVTCLGLSY	176	11		653
YLQLVFGI	157	8		654
YVKVLHHTL	283	9		655
YVKVLHHTLKI	283	11		656

Table X B
Mage 3 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
ALSRKVAEL	108	9		657
ALVETSYVKVL	277	11		658
ATCLGLSY	179	8		659
ATCLGLSYDGL	179	11		660
CLGLSYDGL	181	9		661
CLGLSYDGLL	181	10		662
CYEFLWGPRAL	268	11	0.0004	663
DLESEFQAAL	100	10		664
EFLWGPRAL	270	9	0.0006	665
ELMEVDPI	165	8		666
ELMEVDPIGHL	165	11		667
ELSVLEVF	224	8		668
ELVHFLLL	115	8		669
ELVHFLLLKY	115	10		670
EMLGSVVGNW	134	10	0.0017	671
ETSYVKVL	280	8		672
ETSYVKVLHHM	280	11		673
EVDPIGHL	168	8		674
EVDPIGHLY	168	9		675
EVDPIGHLYI	168	10		676
EVDPIGHLYIF	168	11		677
EVFEGREDSI	229	10		678
EVFEGREDSIL	229	11		679
FLWGPRAL	271	8		680
FVQENYLEY	250	9		681
GIELMEVDPI	163	10		682
GLEARGEAL	15	9		683
GLEARGEALGL	15	11		684
GLLGDNQI	188	8		685
GLLGDNQIM	188	9		686
GLLHVLAI	200	9		687
GLLHVLAII	200	10		688
GLSYDGLL	183	8		689
HFVQENYL	249	8	-0.0004	690
HFVQENYLEY	249	10		691
HHSYPPLHEW	298	10		692
HLYIFATCL	174	9		693
HLYIFATCLGL	174	11		694
HMVKISGGPHI	289	11		695
IFATCLGL	177	8	0 0120	696
IFATCLGLSY	177	10		697
IFSKASSSL	150	9	0 0160	698
IFSKASSSLQL	150	11	0 0910	699
ILGDPKKL	238	8		700
ILGDPKKLL	238	9		701
IMPKAGLL	195	8		702
IMPKAGLLI	195	9	0 4200	703
IMPKAGLLII	195	10	0.0500	704
IWEELSVL	221	8	-0.0004	705
IWEELSVLEVF	221	11	0.0260	706

Table X B

Mage 3 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
KISGGPHI	292	8		707
KISGGPHISY	292	10		708
KIWEELSVL	220	9		709
KVAELVHF	112	8		710
KVAELVHFL	112	9		711
KVAELVHFLL	112	10		712
KVAELVHFLLL	112	11		713
KVLHHMVKI	285	9		714
LHVLAH	202	8		715
LLGDNQIM	189	8		716
LLHVLAI	201	8		717
LLHVLAH	201	9		718
LLTQHFVQENY	245	11		719
LMEVDPIGHL	166	10		720
LMEVDPIGHLY	166	11		721
LTQHFVQENY	246	10		722
LTQHFVQENYL	246	11		723
LVETSYVKVL	278	10		724
LVFGIELM	160	8		725
LVHFLLLKY	116	9		726
LYIFATCL	175	8	0.0140	727
LYIFATCLGL	175	10	0.0480	728
MLGSVVGNW	135	9		729
MLGSVVGNWQY	135	11		730
MVKISGGPHI	290	10		731
NWQYFFPVI	142	9	0.5300	732
NWQYFFPVIF	142	10	0.0170	733
NYPLWSQSY	76	9	0.0270	734
PIGHLYIF	171	8		735
PTTMNYPL	72	8		736
PTTMNYPLW	72	9		737
PVIFSKASSSL	148	11		738
PVTKAEML	129	8		739
QIMPKAGL	194	8		740
QIMPKAGLL	194	9		741
QIMPKAGLLI	194	10		742
QIMPKAGLLII	194	11		743
QLVFGIEL	159	8		744
QLVFGIELM	159	9		745
QVPGSDPACY	260	10		746
QYFFPVIF	144	8	0.1200	747
SILGDPKKL	237	9		748
SILGDPKKLL	237	10		749
SLPTTMNY	70	8		750
SLPTTMNYPL	70	10		751
SLPTTMNYPLW	70	11		752
SLQLVFGI	157	8		753
SLQLVFGIEL	157	10		754
SLQLVFGIELM	157	11		755
STFPDLESEF	96	10		756

Table X B

Mage 3 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
STLVEVTL	43	8		757
SVVGNWQY	138	8		758
SVVGNWQYF	138	9		759
SVVGNWQYFF	138	10		760
SYDGLLGDNQI	185	11	0.0026	761
SYPPLHEW	300	8	0.0420	762
SYPPLHEWVL	300	10	0.5900	763
SYVKVLHHIM	282	9		764
TFPDLESEF	97	9	0.0049	765
TMNYPLWSQSY	74	11		766
TTMNYPLW	73	8		767
VFEGREDSI	230	9	-0.0004	768
VFEGREDSIL	230	10	-0.0005	769
VIFSKASSSL	149	10		770
VLHHMVKI	286	8		771
VVGNWQYF	139	8		772
VVGNWQYFF	139	9		773
YIFATCLGL	176	9		774
YIFATCLGLSY	176	11		775
YVKVLHHIM	283	8		776
YVKVLHHIMVKI	283	11		777

Table XI A

Mage 2 B07 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
APATEEQQTA	30	10	0.0002	778
APEEKIWEEL	216	10	0.0001	779
DPACYEFL	265	8	-0.0002	780
DPACYEFLW	265	9	0.0001	781
EPHHSYPPL	296	9	0.1100	782
EPVTKAEM	128	8	0.0010	783
EPVTKAEML	128	9	0.0001	784
FPDLESEF	98	8	-0.0002	785
FPDLESEFQA	98	10	0.0002	786
FPDLESEFQAA	98	11	-0 0001	787
FPVIFSKA	147	8	0.0003	788
FPVIFSKASEY	147	11	0.0004	789
GPRALIETSY	274	10	0.0008	790
GPRALIETSYV	274	11	0.1300	791
GPRMFPDL	94	8	0.0063	792
HPRKLLMQDL	241	10	0.0400	793
HPRKLLMQDLV	241	11	0.0042	794
KPEEGLEA	11	8	-0 0002	795
MPKTGLLI	196	8	0 0190	796
MPKTGLLIH	196	9	0.0020	797
MPKTGLLIHV	196	10	0.0003	798
MPKTGLLIHVL	196	11	0.0099	799
PPHSPQGA	61	8	-0 0002	800
PPHSPQGASSF	61	11	-0 0003	801
PPLHERAL	302	8	0 0026	802
SPPHSPQGA	60	9	0.0001	803
SPQGASSF	64	8	0 0007	804
SPSPPHSPQGA	58	11	0 0006	805
VPGSDPACY	261	9	0 0001	806
VPGSDPACYEF	261	11	-0.0001	807
VPISHLYI	170	8	0.0170	808
VPISHLYIL	170	9	0.2500	809
VPISHLYILV	170	10	0 0027	810
YPPLHERA	301	8	-0 0002	811
YPPLHERAL	301	9	0 2700	812

Table XI B

Mage 3 B07 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
APATEEQEA	30	9	0.0001	813
APATEEQEAA	30	10	0.0002	814
APEEKIWEEL	216	10	0.0001	815
DPACYEFL	265	8	-0.0002	816
DPACYEFLW	265	9	0.0001	817
DPIGHLYI	170	8	-0.0002	818
DPIGHLYIF	170	9	0.0001	819
DPIGHLYIFA	170	10	0.0002	820
DPKKLLTQHF	241	10	0.0001	821
DPKKLLTQHFV	241	11	-0.0004	822
DPPQSPQGA	60	9	0 0001	823
EPVTKAEM	128	8	0.0010	824
EPVTKAEML	128	9	0.0001	825
FPDLESEF	98	8	-0.0002	826
FPDLESEFQA	98	10	0.0002	827
FPDLESEFQAA	98	11	-0.0001	828
FPVIFSKA	147	8	0.0003	829
GPHISYPPL	296	9	0.8800	830
GPRALVETSY	274	10	0.0002	831
GPRALVETSYV	274	11	0.1900	832
GPSTFPDL	94	8	-0.0002	833
KPEEGLEA	11	8	-0.0002	834
LPTTMNYPL	71	9	0.0770	835
LPTTMNYPLW	71	10	0.0001	836
MPKAGLLI	196	8	0.1300	837
MPKAGLLII	196	9	0.0170	838
MPKAGLLIIV	196	10	0.0031	839
MPKAGLLIIVL	196	11	0.0280	840
PPLHEWVL	302	8	-0 0002	841
PPQSPQGA	61	8	-0.0002	842
PPQSPQGASSL	61	11	0.0049	843
SPDPPQSPQGA	58	11	-0 0001	844
SPQGASSL	64	8	0.0081	845
VPGSDPACY	261	9	0.0001	846
VPGSDPACYEF	261	11	-0 0001	847
YPLWSQSY	77	8	-0 0002	848
YPPLHEWV	301	8	-0.0002	849
YPPLHEWVL	301	9	0 0027	850

Table XII A
Mage 2 B27 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AHPRKLLM	240	8	851
AHPRKLLMQDL	240	11	852
AREPVTKAEM	126	10	853
AREPVTKAEML	126	11	854
ARGEALGL	18	8	855
EKIWEELSM	219	9	856
EKIWEELSML	219	10	857
LKIGGEPHI	291	9	858
LKIGGEPHISY	291	11	859
LRNCQDFF	140	8	860
LRNCQDFFPVI	140	11	861
PHISYPPL	297	8	862
PHSPQGASSF	62	10	863
PKTGLLII	197	8	864
PKTGLLIIVL	197	10	865
PRALIETSY	275	9	866
PRKLLMQDL	242	9	867
PRMFPDLESEF	95	11	868
QHCKPEEGL	8	9	869
RKLLMQDL	243	8	870
RKMVELVHF	111	9	871
RKMVELVHFL	111	10	872
RKMVELVHFLL	111	11	873
SHLYILVTCL	173	10	874
SKASEYLQL	152	9	875
SKASEYLQLVF	152	11	876
SRKMVELVHF	110	10	877
SRKMVELVHFL	110	11	878
TKAEMLESVL	131	10	879
VHFLLLKY	117	8	880
VKVLHHTL	284	8	881
VKVLHHTLKI	284	10	882

Table XII B
Mage 3 B27 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AREPVTKAEM	126	10	883
AREPVTKAEML	126	11	884
ARGEALGL	18	8	885
EKIWEELSVL	219	10	886
GHLYIFATCL	173	10	887
KKLLTQHF	243	8	888
PHISYPPL	297	8	889
PHISYPPLHEW	297	11	890
PKAGLLH	197	8	891
PKAGLLHVL	197	10	892
PKKLLTQHF	242	9	893
PRALVETSY	275	9	894
QHCKPEEGL	8	9	895
QHFVQENY	248	8	896
QHFVQENYL	248	9	897
QHFVQENYLEY	248	11	898
RKVAELVHF	111	9	899
RKVAELVHFL	111	10	900
RKVAELVHFLL	111	11	901
SKASSSLQL	152	9	902
SKASSSLQLVF	152	11	903
SRKVAELVHF	110	10	904
SRKVAELVHFL	110	11	905
VHFLLLKY	117	8	906
VKISGGPHI	291	9	907
VKISGGPHISY	291	11	908
VKVLHHMVKI	284	10	909

Table XIII A

Mage 2 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AAISRKMV	107	8	910
AAISRKMVEL	107	10	911
AAISRKMVELV	107	11	912
ASEYLQLV	154	8	913
ASEYLQLVF	154	9	914
ASEYLQLVFGI	154	11	915
ASSFSTTI	68	8	916
ASSFSTTINY	68	10	917
ASSSSTLV	39	8	918
ASSSSTLVEV	39	10	919
CAPEEKIW	215	8	920
CAPEEKIWEEL	215	11	921
DSVFAHPRKL	236	10	922
DSVFAHPRKLL	236	11	923
EARGEALGL	17	9	924
EARGEALGLV	17	10	925
ESEFQAAI	102	8	926
ESVLRNCQDF	137	10	927
ESVLRNCQDFF	137	11	928
ETSYVKVL	280	8	929
FAHPRKLL	239	8	930
FAHPRKLLM	239	9	931
FSKASEYL	151	8	932
FSKASEYLQL	151	10	933
FSKASEYLQLV	151	11	934
FSTTINYTL	71	9	935
FSTTINYTLW	71	10	936
GASSFSTTI	67	9	937
GASSFSTTINY	67	11	938
GSDPACYEF	263	9	939
GSDPACYEFL	263	10	940
GSDPACYEFLW	263	11	941
HSPQGASSF	63	9	942
HTLKIGGEPHI	289	11	943
ISHLYILV	172	8	944
ISHLYILVTCL	172	11	945
ISRKMVEL	109	8	946
ISRKMVELV	109	9	947
ISRKMVELVHF	109	11	948
ISYPPLHERAL	299	11	949
KAEMLESV	132	8	950
KAEMLESVL	132	9	951
KASEYLQL	153	8	952
KASEYLQLV	153	9	953
KASEYLQLVF	153	10	954
KTGLLHV	198	8	955
KTGLLHVL	198	9	956
KTGLLHVLAI	198	11	957
PACYEFLW	266	8	958
QAAISRKM	106	8	959

Table XIII A
Mage 2 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
QAAISRKMV	106	9	960
QAAISRKMVEL	106	11	961
QTASSSSTL	37	9	962
QTASSSSTLV	37	10	963
RALIETSY	276	8	964
RALIETSYV	276	9	965
RALIETSYVKV	276	11	966
RAREPVTKAEM	125	11	967
RSQIICKPEEGL	6	11	968
SSFSTTINY	69	9	969
SSFSTTINYTL	69	11	970
SSNQEEEGPRM	87	11	971
SSSSTLVEV	40	9	972
SSSSTLVEVTL	40	11	973
SSSTLVEV	41	8	974
SSSTLVEVTL	41	10	975
SSTLVEVTL	42	9	976
STLVEVTL	43	8	977
STLVEVTLGEV	43	11	978
STTINYTL	72	8	979
STTINYTLW	72	9	980
TASSSSTL	38	8	981
TASSSSTLV	38	9	982
TASSSSTLVEV	38	11	983
TSYVKVLHHTL	281	11	984
TTINYTLW	73	8	985
VTCLGLSY	179	8	986
VTCLGLSYDGL	179	11	987
VTKAEMLESV	130	10	988
VTKAEMLESVL	130	11	989

Table XIII B
Mage 3 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AALSRKVAEL	107	10	990
AALSRKVAELV	107	11	991
AASSSSTL	38	8	992
AASSSSTLV	38	9	993
AASSSSTLVEV	38	11	994
ASSLPTTM	68	8	995
ASSLPTTMNY	68	10	996
ASSSLQLV	154	8	997
ASSSLQLVF	154	9	998
ASSSLQLVFGI	154	11	999
ASSSSTLV	39	8	1000
ASSSSTLVEV	39	10	1001
ATCLGLSY	179	8	1002
ATCLGLSYDGL	179	11	1003
CAPEEKIW	215	8	1004
CAPEEKIWEEL	215	11	1005
DSILGDPKKL	236	10	1006
DSILGDPKKLL	236	11	1007
EAASSSSTL	37	9	1008
EAASSSSTLV	37	10	1009
EARGEALGL	17	9	1010
EARGEALGLV	17	10	1011
ESEFQAAL	102	8	1012
ETSYVKVL	280	8	1013
ETSYVKVLHHIM	280	11	1014
FATCLGLSY	178	9	1015
FSKASSSL	151	8	1016
FSKASSSLQL	151	10	1017
FSKASSSLQLV	151	11	1018
GASSLPTTM	67	9	1019
GASSLPTTMNY	67	11	1020
GSDPACYEF	263	9	1021
GSDPACYEFL	263	10	1022
GSDPACYEFLW	263	11	1023
GSVVGNWQY	137	9	1024
GSVVGNWQYF	137	10	1025
GSVVGNWQYFF	137	11	1026
ISGGPHISY	293	9	1027
ISYPPLHEW	299	9	1028
ISYPPLHEWV	299	10	1029
ISYPPLHEWVL	299	11	1030
KAEMLGSV	132	8	1031
KAEMLGSVV	132	9	1032
KAGLLHV	198	8	1033
KAGLLHVL	198	9	1034
KAGLLHVLAI	198	11	1035
KASSSLQL	153	8	1036
KASSSLQLV	153	9	1037
KASSSLQLVF	153	10	1038
LSRKVAEL	109	8	1039

Table XIII B

Mage 3 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LSRKVAELV	109	9	1040
LSRKVAELVHF	109	11	1041
LTQHFVQENY	246	10	1042
LTQHFVQENYL	246	11	1043
PACYEFLW	266	8	1044
PSTFPDLESEF	95	11	1045
PTTMNYPL	72	8	1046
PTTMNYPLW	72	9	1047
QAALSRKV	106	8	1048
QAALSRKVAEL	106	11	1049
QSPQGASSL	63	9	1050
RALVETSY	276	8	1051
RALVETSYV	276	9	1052
RALVETSYVKV	276	11	1053
RAREPVTKAEM	125	11	1054
RSQHCKPEEGL	6	11	1055
SSLPTTMNY	69	9	1056
SSLPTTMNYPL	69	11	1057
SSLQLVFGI	156	9	1058
SSLQLVFGIEL	156	11	1059
SSSLQLVF	155	8	1060
SSSLQLVFGI	155	10	1061
SSSSTLVEV	40	9	1062
SSSSTLVEVTL	40	11	1063
SSSTLVEV	41	8	1064
SSSTLVEVTL	41	10	1065
SSTLVEVTL	42	9	1066
STFPDLESEF	96	10	1067
STLVEVTL	43	8	1068
STLVEVTLGEV	43	11	1069
TSYVKVLHHM	281	10	1070
TSYVKVLHHMV	281	11	1071
TTMNYPLW	73	8	1072
VAELVHFL	113	8	1073
VAELVHFLL	113	9	1074
VAELVHFLLL	113	10	1075
VTKAEMLGSV	130	10	1076
VTKAEMLGSVV	130	11	1077

Table XIV A
Mage 2 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AISRKMVELV	108	10	1078
ALIETSYV	277	8	1079
ALIETSYVKV	277	10	1080
CQDFFPVI	143	8	1081
CQDFFPVIF	143	9	1082
DLESEFQAAI	100	10	1083
DLVQENYLEY	249	10	1084
DPACYEFLW	265	9	1085
ELSMLEVF	224	8	1086
ELVHFLLLKY	115	10	1087
EPVTKAEM	128	8	1088
EVFEGREDSV	229	10	1089
EVFEGREDSVF	229	11	1090
EVVEVVPI	165	8	1091
EVVPISHLY	168	9	1092
EVVPISHLYI	168	10	1093
FLWGPRALI	271	9	1094
FPDLESEF	98	8	1095
FPVIFSKASEY	147	11	1096
FQAAISRKM	105	9	1097
FQAAISRKMV	105	10	1098
GIEVVEVV	163	8	1099
GIEVVFVVPI	163	10	1100
GLLGDNQV	188	8	1101
GLLGDNQVM	188	9	1102
GLLHVLAI	200	9	1103
GLLHVLAII	200	10	1104
GPRALIETSY	274	10	1105
GPRALIETSYV	274	11	1106
HPRKLLMQDLV	241	11	1107
HVLAHAI	203	9	1108
HLVTCLGLSY	177	10	1109
IVLAHAI	204	8	1110
KIGGEPHI	292	8	1111
KIGGEPHISY	292	10	1112
KIWEELSM	220	8	1113
KIWEELSMLEV	220	11	1114
KLLMQDLV	244	8	1115
KMVELVHF	112	8	1116
KVLHHTLKI	285	9	1117
LIETSYVKV	278	9	1118
LHVLAII	202	8	1119
LHVLAHAI	202	10	1120
LLGDNQVM	189	8	1121
LLHVLAI	201	8	1122
LLHVLAII	201	9	1123
LLHVLAHAI	201	11	1124
LLKYRAREPV	121	10	1125
LLLKYRAREPV	120	11	1126
LLMQDLVQENY	245	11	1127

Table XIV A
Mage 2 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LMQDLVQENY	246	10	1128
LQLVFGIEV	158	9	1129
LQLVFGIEVV	158	10	1130
LVEVTLGEV	45	9	1131
LVFGIEVV	160	8	1132
LVFGIEVVEV	160	10	1133
LVFGIEVVEVV	160	11	1134
LVIIFLLLKY	116	9	1135
LVQENYLEY	250	9	1136
LVTCLGLSY	178	9	1137
MPKTGLLI	196	8	1138
MPKTGLLII	196	9	1139
MPKTGLLIIV	196	10	1140
MQDLVQENY	247	9	1141
NQEEEGPRM	89	9	1142
NQEEEGPRMF	89	10	1143
NQVMPKTGLLI	193	11	1144
PISHLYILV	171	9	1145
PPHSPQGASSF	61	11	1146
PQGASSFSTTI	65	11	1147
PVIFSKASEY	148	10	1148
PVTKAEMLESV	129	11	1149
QLVFGIEV	159	8	1150
QLVFGIEVV	159	9	1151
QLVFGIEVVEV	159	11	1152
QQTASSSSTLV	36	11	1153
QVMPKTGLLI	194	10	1154
QVMPKTGLLII	194	11	1155
QVPGSDPACY	260	10	1156
RMFPDLESEF	96	10	1157
RQVPGSDPACY	259	11	1158
SPQGASSF	64	8	1159
SVFAHPRKLLM	237	11	1160
SVLRNCQDF	138	9	1161
SVLRNCQDFF	138	10	1162
TLKIGGEPHI	290	10	1163
TLVEVTLGEV	44	10	1164
VIFSKASEY	149	9	1165
VLHIITLKI	286	8	1166
VLRNCQDF	139	8	1167
VLRNCQDFF	139	9	1168
VLRNCQDFFPV	139	11	1169
VMPKTGLLI	195	9	1170
VMPKTGLLII	195	10	1171
VMPKTGLLIIV	195	11	1172
VPGSDPACY	261	9	1173
VPGSDPACYEF	261	11	1174
VPISHLYI	170	8	1175
VPISHLYILV	170	10	1176
VQENYLEY	251	8	1177

Table XIV A
Mage 2 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
VQENYLEYRQV	251	11	1178
VVEVVPISHLY	166	11	1179
VVPISHLY	169	8	1180
VVPISHLYI	169	9	1181
VVPISHLYILV	169	11	1182
YILVTCLGLSY	176	11	1183
YLQLVFGI	157	8	1184
YLQLVFGIEV	157	10	1185
YLQLVFGIEVV	157	11	1186
YVKVLHHTLKI	283	11	1187

Table XIV B

Mage 3 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO
ALSRKVAELV	108	10	1188
ALVETSYV	277	8	1189
ALVETSYVKV	277	10	1190
DPACYEFLW	265	9	1191
DPIGHLYI	170	8	1192
DPIGHLYIF	170	9	1193
DPKKLLTQHF	241	10	1194
DPKKLLTQHFV	241	11	1195
ELMEVDPI	165	8	1196
ELSVLEVF	224	8	1197
ELVHFLLLKY	115	10	1198
EMLGSVVGNW	134	10	1199
EPVTKAEM	128	8	1200
EVDPIGHLY	168	9	1201
EVDPIGHLYI	168	10	1202
EVDPIGHLYIF	168	11	1203
EVFEGREDSI	229	10	1204
FLWGPRALV	271	9	1205
FPDLESEF	98	8	1206
FQAALSRKV	105	9	1207
FVQENYLEY	250	9	1208
GIELMEVDPI	163	10	1209
GLLGDNQI	188	8	1210
GLLGDNQIM	188	9	1211
GLLIIVLAI	200	9	1212
GLLIIVLAII	200	10	1213
GPRALVETSY	274	10	1214
GPRALVETSYV	274	11	1215
HISYPPLHEW	298	10	1216
HISYPPLHEWV	298	11	1217
HMVKISGGPHI	289	11	1218
IMPKAGLLI	195	9	1219
IMPKAGLLII	195	10	1220
IMPKAGLLIIV	195	11	1221
KISGGPHI	292	8	1222
KISGGPHISY	292	10	1223
KIWEELSV	220	8	1224
KIWEELSVLEV	220	11	1225
KLLTQHFV	244	8	1226
KVAELVHF	112	8	1227
KVLHHMVKI	285	9	1228
LIIVLAII	202	8	1229
LLGDNQIM	189	8	1230
LLIIVLAI	201	8	1231
LLIIVLAII	201	9	1232
LLKYRAREPV	121	10	1233
LLLKYRAREPV	120	11	1234
LLTQHFVQENY	245	11	1235
LMEVDPIGHLY	166	11	1236
LPTTMNYPLW	71	10	1237

Table XIV B
Mage 3 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LQLVFGIELM	158	10	1238
LVETSYVKV	278	9	1239
LVEVTLGEV	45	9	1240
LVFGIELM	160	8	1241
LVFGIELMEV	160	10	1242
LVIIFLLLKY	116	9	1243
MLGSVVGNW	135	9	1244
MLGSVVGNWQY	135	11	1245
MPKAGLLI	196	8	1246
MPKAGLLII	196	9	1247
MPKAGLLIIV	196	10	1248
MVKISGGPII	290	10	1249
NQEEEGPSTF	89	10	1250
NQIMPKAGLLI	193	11	1251
PIGIILYIF	171	8	1252
PQGASSLPTTM	65	11	1253
PVTKAEMLGSV	129	11	1254
QIMPKAGLLI	194	10	1255
QIMPKAGLLII	194	11	1256
QLVFGIELM	159	9	1257
QLVFGIELMEV	159	11	1258
QVPGSDPACY	260	10	1259
RQVPGSDPACY	259	11	1260
SLPTTMNY	70	8	1261
SLPTTMNYPLW	70	11	1262
SLQLVFGI	157	8	1263
SLQLVFGIELM	157	11	1264
SVVGNWQY	138	8	1265
SVVGNWQYF	138	9	1266
SVVGNWQYFF	138	10	1267
TLVEVTLGEV	44	10	1268
TMNYPLWSQSY	74	11	1269
TQIIFVQENY	247	9	1270
VLIIIIMVKI	286	8	1271
VPGSDPACY	261	9	1272
VPGSDPACYEF	261	11	1273
VQENYLEY	251	8	1274
VQENYLEYRQV	251	11	1275
VVGNWQYF	139	8	1276
VVGNWQYFF	139	9	1277
VVGNWQYFFPV	139	11	1278
WQYFFPVI	143	8	1279
WQYFFPVIF	143	9	1280
YIFATCLGLSY	176	11	1281
YPLWSQSY	77	8	1282
YPPLIIEWV	301	8	1283
YVKVLIIIM	283	8	1284
YVKVLIIIMV	283	9	1285
YVKVLIIIMVKI	283	11	1286

Table XV A
Mage 2 A01 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASSFSTTINY	68	10	0.1700	1287
GASSFSTTINY	67	11	0.0047	1288
GGEPHISY	294	8	-0.0021	1289
IFSKASEY	150	8	0.0023	1290
LMQDLVQENY	246	10	0.0450	1291
MQDLVQENY	247	9	1.5000	1292
PGSDPACY	262	8	-0.0021	1293
PRALIETSY	275	9	-0.0006	1294
SFSTTINY	70	8	-0.0021	1295
SSFSTTINY	69	9	0.0430	1296
VQENYLEY	251	8	-0.0021	1297
VTCLGLSY	179	8		1298
VVEVVPISHLY	166	11	0.2000	1299

Table XV B

Mage 3 A01 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASSLPTTMNY	68	10	2 6000	1300
ATCLGLSY	179	8	0.1100	1301
EVDPIGHLY	168	9	18.0000	1302
GASSLPTTMNY	67	11	0.0390	1303
GSVVGNWQY	137	9	0 0500	1304
IFATCLGLSY	177	10	0.0020	1305
ISGGPHISY	293	9	0.0370	1306
KISGGPHISY	292	10	0.0011	1307
LGSVVGNWQY	136	10	0.0020	1308
LMEVDPIGHLY	166	11	7.5000	1309
LTQHFVQENY	246	10	0.2600	1310
PGSDPACY	262	8	-0.0021	1311
PRALVETSY	275	9	0.0011	1312
SSLPTTMNY	69	9	0.0550	1313
TMNYPLWSQSY	74	11	0.0830	1314
VQENYLEY	251	8	-0.0021	1315

Table XVI A

Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO
AADSPSPPH	55	9	0.0003	1316
ACYEFLWGPR	267	10	0.0032	1317
ACYEFLWGPRA	267	11		1318
ADSPSPPH	56	8		1319
AIEGDCAPEEK	210	11	0.0009	1320
AIIAIEGDCA	207	10		1321
AISRKMVELVII	108	11		1322
ALGLVGAQA	22	9	0.0003	1323
ALGLVGAQAPA	22	11		1324
ALIETSYVK	277	9	0.0810	1325
ASEYLQLVF	154	9	0.0002	1326
ASSFSTTINY	68	10	0.0009	1327
ATEEQQTA	32	8		1328
DFFPVIFSK	145	9	0.0002	1329
DFFPVIFSKA	145	10		1330
DLESEFQA	100	8		1331
DLESEFQAA	100	9		1332
DLVQENYLEY	249	10		1333
DLVQENYLEYR	249	11	0.0047	1334
DSVFAIIPR	236	8	-0.0004	1335
DSVFAIIPRK	236	9	0.0021	1336
EALGLVGA	21	8		1337
EALGLVGAQA	21	10	0 0003	1338
EDSVFAIIPR	235	9		1339
EDSVFAIIPRK	235	10		1340
EFLWGPRA	270	8		1341
EFQAAISR	104	8		1342
EFQAAISRK	104	9	0 0002	1343
EGDCAPEEK	212	9	0 0002	1344
EGLEARGEA	14	9	0 0003	1345
EGREDSVF	232	8		1346
EGREDSVFA	232	9		1347
EGREDSVFAH	232	10		1348
ELSMLEVF	224	8		1349
ELSMLEVFEGR	224	11	0 0016	1350
ELVHFLLLK	115	9	0 0045	1351
ELVHFLLLKY	115	10	0 0066	1352
ELVHFLLLKYR	115	11	0 0011	1353
EMLESVLR	134	8	-0 0009	1354
ESEFQAAISR	102	10	0.0002	1355
ESEFQAAISRK	102	11	0 0010	1356
ESVLRNCQDF	137	10	0 0002	1357
ESVLRNCQDFF	137	11		1358
ETSYVKVLH	280	9		1359
ETSYVKVLHH	280	10		1360
EVFEGREDSVF	229	11		1361
EVTLGEVPA	47	9	0 0003	1362
EVTLGEVPAA	47	10	0 0003	1363
EVVEVVPISH	165	10	0.0002	1364
EVVPISHLY	168	9	0.0002	1365

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Table XVI A

Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
FFPVIFSK	146	8		1366
FFPVIFSKA	146	9	0.0003	1367
FLLLKYRA	119	8		1368
FLLLKYRAR	119	9		1369
FSTTINYTLWR	71	11	0.0110	1370
GASSFSTTINY	67	11		1371
GDCAPEEK	213	8		1372
GDNQVMPK	191	8		1373
GGFPHISY	294	8		1374
GLEARGEA	15	8		1375
GLLGDNQVMPK	188	11	0.0780	1376
GLLHVLA	200	8		1377
GLLHVLAHA	200	11		1378
GLVGAQAPA	24	9	0.0003	1379
GSDPACYEF	263	9		1380
GSSNQEEEGPR	86	11	-0.0002	1381
HCKPEEGLEA	9	10	0.0003	1382
HCKPEEGLEAR	9	11		1383
HFLLLKYR	118	8		1384
HFLLLKYRA	118	9	0.0016	1385
HFLLLKYRAR	118	10	0.0014	1386
HISYPPLH	298	8		1387
HISYPPLHER	298	10	0.0074	1388
HISYPPLHERA	298	11		1389
HSPQGASSF	63	9	0.0002	1390
HTLKIGGEPH	289	10		1391
IAIEGDCA	209	8		1392
IFSKASEY	150	8		1393
IGGEPHISY	293	9		1394
HAIEGDCA	208	9		1395
HVLAHA	203	8		1396
ILVTCLGLSY	177	10	0.0036	1397
ISRKMVELVH	109	10	0.0002	1398
ISRKMVELVHF	109	11		1399
ISYPPLHER	299	9	0.0340	1400
ISYPPLHERA	299	10		1401
KAEMLESVLR	132	10	0.0002	1402
KASEYLQLVF	153	10	0.0002	1403
KIGGEPHISY	292	10		1404
KMVELVHF	112	8		1405
KTGLLHVLA	198	10		1406
KVLHHTLK	285	8	0.0053	1407
LAHAIEGDCA	206	11		1408
LGDNQVMPK	190	9	0.0002	1409
LGLVGAQA	23	8		1410
LGLVGAQAPA	23	10	0.0003	1411
LIETSYVK	278	8	-0.0004	1412
LIETSYVKVLH	278	11		1413
LHVLAHA	202	9		1414
LLGDNQVMPK	189	10	0.0093	1415

Table XVI A

Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
LLIIVLAIIA	201	10		1416
LLLKYRAR	120	8	-0.0009	1417
LLMQDLVQENY	245	11		1418
LMQDLVQENY	246	10		1419
LSMLEVFEGR	225	10	-0.0004	1420
LVEVTLGEVPA	45	11		1421
LVGAQAPA	25	8		1422
LVIIFLLLK	116	8	0.0290	1423
LVIIFLLLKY	116	9	0.0430	1424
LVIIFLLLKYR	116	10	0.0260	1425
LVIIFLLLKYRA	116	11		1426
LVQENYLEY	250	9		1427
LVQENYLEYR	250	10	0.0027	1428
LVTCLGLSY	178	9		1429
MFPDLESEF	97	9	0.0002	1430
MFPDLESEFQA	97	11		1431
MLEVFEGR	227	8	-0.0009	1432
MVELVIIFLLLK	113	11	0.0200	1433
NCQDFFPVIF	142	10	0.0002	1434
PAADSPSPPII	54	10		1435
PACYEFLWGPR	266	11	-0.0009	1436
PATEEQQTA	31	9		1437
PDLESEFQA	99	9	0.0003	1438
PDLESEFQAA	99	10	0.0003	1439
PGSDPACY	262	8		1440
PGSDPACYEF	262	10		1441
PLEQRSQII	2	8		1442
PLEQRSQHCK	2	10	0.0003	1443
PLIIERALR	303	8	-0.0009	1444
PSPPIISPQGA	59	10		1445
PVIFSKASEY	148	10	0.0160	1446
QAPATEEQQTA	29	11		1447
QDFFPVIF	144	8		1448
QDFFPVIFSK	144	10	0.0002	1449
QDFFPVIFSKA	144	11		1450
QDLVQENY	248	8		1451
QDLVQENYLEY	248	11		1452
QVPGSDPA	260	8		1453
QVPGSDPACY	260	10		1454
RALIETSY	276	8		1455
RALIETSYVK	276	10	0 0200	1456
RAREPVTK	125	8	-0 0009	1457
RAREPVTKA	125	9		1458
RGEALGLVGA	19	10	0 0003	1459
RMFPDLESEF	96	10	0 0002	1460
SDPACYEF	264	8		1461
SFSTTINY	70	8		1462
SMLEVFEGR	226	9	0.0020	1463
SSFSTTINY	69	9		1464
SSNQEEEGPR	87	10	0.0002	1465

Table XVI A

Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
STIINYTLWR	72	10	0.0014	1466
SVFAIIPRK	237	8	0.1410	1467
SVLRNCQDF	138	9	0.0002	1468
SVLRNCQDFF	138	10	0.0002	1469
TGLLIIVLA	199	9		1470
TINYTLWR	74	8	0.0140	1471
TLGEVPAA	49	8		1472
TLKIGGEPH	290	9		1473
TSYVKVLH	281	8		1474
TSYVKVLHH	281	9	0.5900	1475
TTINYTLWR	73	9	0.0890	1476
VFEGREDSVF	230	10		1477
VFEGREDSVFA	230	11		1478
VIFSKASEY	149	9	0.0810	1479
VLRNCQDF	139	8		1480
VLRNCQDFF	139	9	0.0002	1481
VTCLGLSY	179	8		1482
VILGEVPA	48	8		1483
VTLGEVPAA	48	9	0 0003	1484
VVEVVPISH	166	9	0.0007	1485
VVEVVPISHLY	166	11		1486
VVPISHLY	169	8		1487
WGPRALIETSY	273	11		1488
YILVTCLGLSY	176	11		1489
YVKVLHHTLK	283	10	0 0033	1490

Table XVI B

Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
AALSRKVA	107	8		1491
ACYEFLWGPR	267	10	0.0032	1492
ACYEFLWGPRA	267	11		1493
AGLLIIVLA	199	9	0.0006	1494
AIIAREGDCA	207	10		1495
ALGLVGAQA	22	9	0.0003	1496
ALGLVGAQAPA	22	11		1497
ALSRKVAELVII	108	11		1498
ALVETSYVK	277	9	0.0270	1499
ASSLPTTMNY	68	10	0.0009	1500
ASSSLQLVF	154	9	0.0011	1501
ATCLGLSY	179	8		1502
ATEEQEAA	32	8		1503
DLESEFQA	100	8		1504
DLESEFQAA	100	9		1505
DSILGDPK	236	8	-0.0004	1506
DSILGDPKK	236	9	-0.0003	1507
EALGLVGA	21	8		1508
EALGLVGAQA	21	10	0.0003	1509
EDSILGDPK	235	9	0 0003	1510
EDSILGDPKK	235	10	0.0003	1511
EFLWGPRA	270	8		1512
EFQAALSR	104	8		1513
EFQAALSRK	104	9	0.0002	1514
EFQAALSRKVA	104	11		1515
EGDCAPEEK	212	9	0.0002	1516
EGLEARGEA	14	9	0 0003	1517
ELMEVDPIGH	165	10	0 0003	1518
ELSVLEVF	224	8		1519
ELSVLEVFEGR	224	11	-0.0009	1520
ELVHFLLLK	115	9	0.0045	1521
ELVHFLLLKY	115	10	0.0066	1522
ELVHFLLLKYR	115	11	0.0011	1523
ESEFQAALSR	102	10	0.0002	1524
ESEFQAALSRK	102	11	0.0002	1525
ETSYVKVLH	280	9		1526
ETSYVKVLHH	280	10		1527
EVDPIGHLY	168	9	0.0002	1528
EVDPIGHLYIF	168	11		1529
EVTLGEVPA	47	9	0 0003	1530
EVTLGEVPAA	47	10	0 0003	1531
FAICLGLSY	178	9	0 0003	1532
FFPVIFSK	146	8		1533
FFPVIFSKA	146	9	0 0003	1534
FLLLKYRA	119	8		1535
FLLLKYRAR	119	9		1536
FVQENYLEY	250	9		1537
FVQENYLEYR	250	10	0.0009	1538
GASSLPTTMNY	67	11		1539
GDCAPEEK	213	8		1540

Table XVI B

Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
GDNQIMPK	191	8		1541
GDNQIMPKA	191	9	0.0003	1542
GDPKKLLTQH	240	10	0 0003	1543
GDPKKLLTQHF	240	11		1544
GGPHISYPPLH	295	11		1545
GLEARGEA	15	8		1546
GLLGDNQIMPK	188	11	0.1300	1547
GLLHVLA	200	8		1548
GLLHVLAHA	200	11		1549
GLVGAQAPA	24	9	0.0003	1550
GSDPACYEF	263	9		1551
GSVVGNWQY	137	9		1552
GSVVGNWQYF	137	10	0.0020	1553
GSVVGNWQYFF	137	11		1554
HCKPEEGLEA	9	10	0.0003	1555
HCKPEEGLEAR	9	11		1556
HFLLLKYR	118	8		1557
HFLLLKYRA	118	9	0.0016	1558
HFLLLKYRAR	118	10	0.0014	1559
HFVQENYI EY	249	10		1560
HFVQENYLEYR	249	11		1561
HHSYPPLH	298	8		1562
HMVKISGGPH	289	10		1563
HAREGDCA	209	8		1564
HFATCLGLSY	177	10	0.0005	1565
HGHLYIFA	172	8		1566
HAREGDCA	208	9		1567
HVLAHA	203	8		1568
HVLAHAR	203	9	0.0069	1569
ISGGPHISY	293	9	0.0003	1570
IVLAHAR	204	8	0.0053	1571
KAGLLHVLA	198	10		1572
KASSSLQLVF	153	10	0 0003	1573
KISGGPHISY	292	10		1574
KVAELVHF	112	8		1575
KVLHHMVK	285	8	0 0580	1576
LAHAREGDCA	206	11		1577
LGDNQIMPK	190	9		1578
LGDNQIMPKA	190	10	0 0003	1579
LGDPKKLLTQH	239	11		1580
LGLVGAQA	23	8		1581
LGLVGAQAPA	23	10	0 0003	1582
LGSVVGNWQY	136	10	0 0003	1583
LGSVVGNWQYF	136	11		1584
LHVLAHA	202	9		1585
LHVLAHAR	202	10	0 0280	1586
LLGDNQIMPK	189	10	0.0200	1587
LLGDNQIMPKA	189	11		1588
LLHVLAHA	201	10		1589
LLHVLAHAR	201	11	0.0021	1590

Table XVI B

Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
LLLKYRAR	120	8	-0.0009	1591
LLTQHFVQENY	245	11		1592
LMEVDPIGH	166	9	0.0002	1593
LMEVDPIGHLY	166	11		1594
LSRKVAELVH	109	10	0.0002	1595
LSRKVAELVHF	109	11		1596
LSVLEVFEGR	225	10	-0.0006	1597
LTQHFVQENY	246	10	0.0003	1598
LVETSYVK	278	8	-0.0004	1599
LVETSYVKVLH	278	11		1600
LVEVTLGEVPA	45	11		1601
LVGAQAPA	25	8		1602
LVHFLLLK	116	8	0.0290	1603
LVHFLLLKY	116	9	0 0430	1604
LVHFLLLKYR	116	10	0.0260	1605
LVHFLLLKYRA	116	11		1606
MLGSVVGNWQY	135	11		1607
MVKISGGPH	290	9	0.0003	1608
PACYEFLWGPR	266	11	-0.0009	1609
PATEEQEA	31	8		1610
PATEEQEAA	31	9	0.0003	1611
PDLESEFQA	99	9	0.0003	1612
PDLESEFQAA	99	10	0.0003	1613
PDPPQSPQGA	59	10	0.0003	1614
PGSDPACY	262	8		1615
PGSDPACYEF	262	10		1616
PIGHLYIF	171	8		1617
PIGHLYIFA	171	9		1618
PLEQRSQH	2	8		1619
PLEQRSQHCK	2	10	0.0003	1620
PLHEWVLR	303	8	-0.0009	1621
PSTFPDLESEF	95	11		1622
QAALSRKVA	106	9		1623
QAPATEEQEA	29	10	0.0003	1624
QAPATEEQEAA	29	11		1625
QVPGSDPA	260	8		1626
QVPGSDPACY	260	10		1627
RALVETSY	276	8		1628
RALVETSYVK	276	10	0.0190	1629
RAREPVTK	125	8	-0 0009	1630
RAREPVTKA	125	9		1631
RGEALGLVGA	19	10	0 0003	1632
SDPACYEF	264	8		1633
SGGPHISY	294	8		1634
SILGDPKK	237	8	-0 0009	1635
SLPTTMNY	70	8		1636
SSLPTTMNY	69	9		1637
SSSLQLVF	155	8		1638
STFPDLESEF	96	10	0.0002	1639
SVLEVFEGR	226	9	0.0003	1640

Table XVI B

Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO
SVVGNWQY	138	8		1641
SVVGNWQYF	138	9	0.0002	1642
SVVGNWQYFF	138	10	0.0085	1643
TFPDLESEF	97	9	0.0002	1644
TFPDLESEFQA	97	11		1645
TLGEVPAA	49	8		1646
TMNYPLWSQSY	74	11		1647
TSYVKVLH	281	8		1648
TSYVKVLHH	281	9	0.5900	1649
VAELVHFLLLK	113	11	-0.0002	1650
VDPIGHLY	169	8		1651
VDPIGHLYIF	169	10	0.0003	1652
VDPIGHLYIFA	169	11		1653
VGNWQYFF	140	8		1654
VLEVFEGR	227	8	0.0016	1655
VTLGEVPA	48	8		1656
VTLGEVPAA	48	9	0.0003	1657
VVGNWQYF	139	8		1658
VVGNWQYFF	139	9	0.0022	1659
WGPRALVETSY	273	11		1660
YFFPVIFSK	145	9	0.0020	1661
YFFPVIFSKA	145	10	0.0003	1662
YIFATCLGLSY	176	11		1663
YVKVLHHMVK	283	10	0.0020	1664

Table XVII A

Mage 2 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
AADSPSPPH	55	9	0 0009	1665
ACYEFLWGPR	267	10	0.0035	1666
ADSPSPPH	56	8		1667
AIEGDCAPEEK	210	11	0.0007	1668
AISRKMVELVH	108	11		1669
ALIETSYVK	277	9	0.1900	1670
ASSFSTTINY	68	10	0.0260	1671
DFFPVIFSK	145	9	0.0022	1672
DLVQENYLEY	249	10		1673
DLVQENYLEYR	249	11	0.0018	1674
DSVFAHPR	236	8	0.0005	1675
DSVFAHPRK	236	9	0.0025	1676
EDSVFAHPR	235	9		1677
EDSVFAHPRK	235	10		1678
EFQAAISR	104	8		1679
EFQAAISRK	104	9	0.0002	1680
EGDCAPEEK	212	9	0.0001	1681
EGREDSVFAH	232	10		1682
ELSMLEVFEGR	224	11	0.0008	1683
ELVHFLLLK	115	9	0.0011	1684
ELVHFLLLKY	115	10	0 0003	1685
ELVHFLLLKYR	115	11	0.0031	1686
EMLESVLR	134	8	-0.0003	1687
ESEFQAAISR	102	10	0 0002	1688
ESEFQAAISRK	102	11	0.0004	1689
ETSYVKVLH	280	9		1690
ETSYVKVLHH	280	10		1691
EVVEVVPISH	165	10	0 0002	1692
EVVPISHLY	168	9	0.0002	1693
FFPVIFSK	146	8		1694
FLLLKYRAR	119	9		1695
FSTTINYTLWR	71	11	0 0170	1696
GASSFSTTINY	67	11		1697
GDCAPEEK	213	8		1698
GDNQVMPK	191	8		1699
GGEPHISY	294	8		1700
GLLGDNQVMPK	188	11	0 0047	1701
GSSNQEEEGPR	86	11	-0 0002	1702
HCKPEEGLEAR	9	11		1703
HFLLLKYR	118	8		1704
HFLLLKYRAR	118	10	0 0002	1705
HISYPPLH	298	8		1706
HISYPPLHER	298	10	0 0018	1707
HTLKIGGEPH	289	10		1708
IFSKASEY	150	8		1709
IGGEPHISY	293	9		1710
ILVTCLGLSY	177	10	0 0002	1711
ISRKMVELVH	109	10	0.0002	1712
ISYPPLHER	299	9	0.0280	1713
KAEMLESVLR	132	10	0.0009	1714

Table XVII A

Mage 2 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
KIGGEPIIISY	292	10		1715
KVLIIHTLK	285	8	0.0100	1716
LGDNQVMPK	190	9	0.0061	1717
LIETSYVK	278	8	0.0027	1718
LIETSYVKVLH	278	11		1719
LLGDNQVMPK	189	10	0.0014	1720
LLLKYRAR	120	8	-0.0004	1721
LLMQDLVQENY	245	11		1722
LMQDLVQENY	246	10		1723
LSMLEVFEGR	225	10	0.0001	1724
LVHFLLLK	116	8	0.1500	1725
LVHFLLLKY	116	9	0.0100	1726
LVHFLLLKYR	116	10	0.0022	1727
LVQENYLEY	250	9		1728
LVQENYLEYR	250	10	0.0089	1729
LVTCLGLSY	178	9		1730
MLEVFEGR	227	8	-0.0004	1731
MVELVHFLLLK	113	11	0.0120	1732
PAADSPSPPH	54	10		1733
PACYEFLWGPR	266	11	-0.0002	1734
PGSDPACY	262	8		1735
PLEQRSQH	2	8		1736
PLEQRSQHCK	2	10	0 0002	1737
PLHERALR	303	8	-0 0004	1738
PVIFSKASEY	148	10	0.0033	1739
QDFFPVIFSK	144	10	0 0083	1740
QDLVQENY	248	8		1741
QDLVQENYLEY	248	11		1742
QVPGSDPACY	260	10		1743
RALIETSY	276	8		1744
RALIETSYVK	276	10	0.0750	1745
RAREPVTK	125	8	-0 0003	1746
SFSTTINY	70	8		1747
SMLEVFEGR	226	9	0 0220	1748
SNQEEEGPR	88	9	0 0001	1749
SSFSTTINY	69	9		1750
SSNQEEEGPR	87	10	0 0002	1751
STTINYTLWR	72	10	0 0910	1752
SVFAHPRK	237	8	0 0810	1753
TINYTLWR	74	8	0 0550	1754
TLKIGGEPH	290	9		1755
TSYVKVLH	281	8		1756
TSYVKVLHH	281	9	0 0066	1757
TTINYTLWR	73	9	1 1000	1758
VIFSKASEY	149	9	0 0330	1759
VTCLGLSY	179	8		1760
VVEVVPISH	166	9	0 0100	1761
VVEVVPISHLY	166	11		1762
VVPISHLY	169	8		1763
WGPRALIETSY	273	11		1764

Table XVII A
Mage 2 A11 Motif Peptides with Binding Data

Sequence	Position	No of Amino Acids	A*1101	SEQ ID NO.
YILVTCLGLSY	176	11	0.0160	1765
YVKVLHHTLK	283	10		1766

Table XVII B
Mage 3 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
ACYEFLWGPR	267	10	0.0035	1767
ALSRKVAELVH	108	11		1768
ALVETSYVK	277	9	0.1700	1769
ASSLPTTMNY	68	10	0.0330	1770
ATCLGLSY	179	8		1771
DSILGDPK	236	8	-0.0003	1772
DSILGDPKK	236	9	-0.0002	1773
EDSILGDPK	235	9	0.0002	1774
EDSILGDPKK	235	10	0.0002	1775
EFQAALSR	104	8		1776
EFQAALSRK	104	9	0.0001	1777
EGDCAPEEK	212	9	0 0001	1778
ELMEVDPIGH	165	10	0 0002	1779
ELSVLEVTEGR	224	11	0.0023	1780
ELVHFLLLK	115	9	0.0011	1781
ELVHFLLLKY	115	10	0.0003	1782
ELVHFLLLKYR	115	11	0.0031	1783
ESEFQAALSR	102	10	0.0002	1784
ESEFQAALSRK	102	11	0.0004	1785
ETSYVKVLH	280	9		1786
ETSYVKVLHH	280	10		1787
EVDPIGHLY	168	9	0.0009	1788
FATCLGLSY	178	9	0.0004	1789
FFPVIFSK	146	8		1790
FLLLKYRAR	119	9		1791
FVQENYLEY	250	9		1792
FVQENYLEYR	250	10	0 0012	1793
GASSLPTTMNY	67	11		1794
GDCAPEEK	213	8		1795
GDNQIMPK	191	8		1796
GDPKKLLTQH	240	10	0.0002	1797
GGPHISYPPLH	295	11		1798
GLLGDNQIMPK	188	11	0 0570	1799
GSVVGNWQY	137	9		1800
HCKPEEGLEAR	9	11		1801
HFLLLKYR	118	8		1802
HFLLLKYRAR	118	10	0.0002	1803
HFVQENYLEY	249	10		1804
HFVQENYLEYR	249	11		1805
HISYPPLH	298	8		1806
HMVKISGGPH	289	10		1807
IFATCLGLSY	177	10	0 0004	1808
IIVLAHAR	203	9	0 0011	1809
ISGGPHISY	293	9	0 0002	1810
IVLAHAR	204	8	0 0037	1811
KISGGPHISY	292	10		1812
KVLHHMVK	285	8	0 0190	1813
LGDNQIMPK	190	9		1814
LGDPKKLLTQH	239	11		1815
LGSVVGNWQY	136	10	0 0012	1816

Table XVII B

Mage 3 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
LIIVLAIIAR	202	10	0.0021	1817
LLGDNQIMPK	189	10	0 0110	1818
LLIIVLAIIAR	201	11	0.0056	1819
LLLKYRAR	120	8	-0.0004	1820
LLTQHFVQENY	245	11		1821
LMEVDPIGH	166	9	0.0001	1822
LMEVDPIGHLY	166	11		1823
LSRKVAELVH	109	10	0.0002	1824
LSVLEVFEGR	225	10	0 0030	1825
LTQHFVQENY	246	10	0.0002	1826
LVETSYVK	278	8	0.0014	1827
LVETSYVKVLH	278	11		1828
LVHFLLLK	116	8	0.1500	1829
LVHFLLLKY	116	9	0.0100	1830
LVHFLLLKYR	116	10	0.0022	1831
MLGSVVGNWQY	135	11		1832
MNYPLWSQSY	75	10	0.0002	1833
MVKISGGPH	290	9	0.0002	1834
PACYEFLWGPR	266	11	-0.0002	1835
PGSDPACY	262	8		1836
PLEQRSQH	2	8		1837
PLEQRSQHCK	2	10	0.0002	1838
PLHEWVLR	303	8	-0 0003	1839
QVPGSDPACY	260	10		1840
RALVETSY	276	8		1841
RALVETSYVK	276	10	0 1100	1842
RAREPVTK	125	8	-0.0003	1843
SGGPHISY	294	8		1844
SILGDPKK	237	8	0.0012	1845
SLPTTMNY	70	8		1846
SSLPTTMNY	69	9		1847
SVLEVFEGR	226	9	0 1400	1848
SVVGNWQY	138	8		1849
TMNYPLWSQSY	74	11		1850
TSYVKVLH	281	8		1851
TSYVKVLHH	281	9	0 0066	1852
VAELVHFLLLK	113	11	0 0011	1853
VDPIGHLY	169	8		1854
VLEVFEGR	227	8	0 0005	1855
WGPRALVETSY	273	11		1856
YFFPVIFSK	145	9	0 0270	1857
YIFATCLGLSY	176	11		1858
YVKVLHHMVK	283	10	0 0061	1859

TableXVIIIA

Mage 2 A24 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
CYEFLWGPRAL	268	11	0.0004	1860
EFLWGPRAL	270	9	0.0006	1861
EFLWGPRALI	270	10	0.0097	1862
EYLQLVFGI	156	9	3.5000	1863
IFSKASEYL	150	9	0.0230	1864
IFSKASEYLQL	150	11	0.0950	1865
IWEELSML	221	8	0.0007	1866
IWEELSMLEVF	221	11	0.0170	1867
KMVELVHF	112	8	0.0005	1868
KMVELVHFL	112	9		1869
KMVELVHFLL	112	10		1870
KMVELVHFLLL	112	11		1871
LMQDLVQENYL	246	11		1872
LWGPRALI	272	8	0.1200	1873
LYILVTCL	175	8	0.0086	1874
LYILVTCLGL	175	10	0.0140	1875
MFPDLESEF	97	9	0.0140	1876
RMFPDLESEF	96	10	0.0016	1877
SFSTTINYTL	70	10	0.0150	1878
SFSTTINYTLW	70	11	0.0280	1879
SYPPLHERAL	300	10	0.0003	1880
SYVKVLHHTL	282	10	0.1600	1881
VFAHPRKL	238	8	0.0005	1882
VFAHPRKLL	238	9	0.0006	1883
VFEGREDSVF	230	10	0.0004	1884
VMPKTGLL	195	8	-0 0004	1885
VMPKTGLLI	195	9	0.2300	1886
VMPKTGLLII	195	10	0.0580	1887

Table XVIII B

Mage 3 A24 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
CYEFLWGPRAL	268	11	0.0004	1888
EFLWGPRAL	270	9	0.0006	1889
EMLGSVVGNW	134	10	0.0017	1890
HFVQENYL	249	8	-0.0004	1891
HMVKISGGPHI	289	11		1892
IFATCLGL	177	8	0.0120	1893
IFSKASSSL	150	9	0 0160	1894
IFSKASSSLQL	150	11	0.0910	1895
IMPKAGLL	195	8		1896
IMPKAGLLI	195	9	0.4200	1897
IMPKAGLLII	195	10	0.0500	1898
IWEELSVL	221	8	-0 0004	1899
IWEELSVLEVF	221	11	0 0260	1900
LMEVDPIGHL	166	10		1901
LYIFATCL	175	8	0 0140	1902
LYIFATCLGL	175	10	0.0480	1903
NWQYFFPVI	142	9	0 5300	1904
NWQYFFPVIF	142	10	0.0170	1905
QYFFPVIF	144	8	0 1200	1906
SYDGLLGDNQI	185	11	0 0026	1907
SYPPLHEW	300	8	0.0420	1908
SYPPLHEWVL	300	10	0.5900	1909
TFPDLESEF	97	9	0 0049	1910
VFEGREDSI	230	9	-0.0004	1911
VFEGREDSIL	230	10	-0.0005	1912

Table XIX A

Mage 2 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2wß1	DR2w2ß2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LVGAQAPAT	ALGLVGAQAPATEEQ	24	0.0330				-0.0032				1913
LSYDGLLGD	CLGLSYDGLLGDNQV	183				0.1400					1914
LGDNQVMPK	DGLLGDNQVMPKTGL	189	-0.0005				-0.0032				1915
IWEELSMLE	EEKIWEELSMLEVFE	220				0.0130					1916
WGPRALIET	EFLWGPRALIETSYV	272									1917
WEELSMLEV	EKIWEELSMLEVFEG	221									1918
LEYRQVPGS	ENYLEYRQVPGSDPA	255									1919
ISYPPLHER	EPHISYPPLHERALR	298	-0.0003				-0.0032				1920
FQAAISRKM	ESEFQAAISRKMVEL	104	1.2000	0.0620	1.0000	0.0113	0.1600		0.0270		1921
LGEVPAADS	EVTLGEVPAADSPSP	49									1922
VIFSKASEY	FFPVIFSKASEYLQL	148									1923
IFSKASEYL	FPVIFSKASEYLQLV	149									1924
LGLVGAQAP	GEALGLVGAQAPATE	22									1925
VVEVVPISH	GIEVVEVVPISHLYI	165	0.0084	0.0046	0.0009	0.0036	0.0070		-0.0005		1926
IIVLAIIAI	GLLIIVLAIIAIEGD	202	0.0100				-0.0032				1927
LLKYRAREP	HFLLLKYRAREPVTK	120									1928
ILVTCLGLS	HLYILVTCLGLSYDG	176									1929
VEVVPISHL	IEVVEVVPISHLYIL	166									1930
IEGDCAPEE	IIAIEGDCAPEEKIW	210				0.0660					1931
LAIIAIEGD	IIVLAIIAIEGDCAP	205									1932
LYILVTCLG	ISHLYILVTCLGLSY	174									1933
MLESVLRNC	KAEMLESVLRNCQDF	134									1934
LLIIVLAII	KTGLLIIVLAIIAIE	200	0.0120	0.0037	-0.0022	0.0025	0.0370		-0.0005		1935
VPAADSPSP	LGEVPAADSPSPPHS	52	-0.0005				-0.0032				1936
VGAQAPATE	LGLVGAQAPATEEQ	25									1937
VLAIIAIEG	LIIVLAIIAIEGDCA	204	0.0120				0.0051				1938
IVLAIIAIE	LLIIVLAIIAIEGDC	203	0.0086				0.0120				1939
YRAREPVTK	LLKYRAREPVTKAEM	123									1940
VFGIEVVEV	LQLVFGIEVVEVVPI	160									1941
VTLGEVPAA	LVEVTLGEVPAADSP	47									1942
LVHFLLLKY	MVELVHFLLLKYRAR	115									1943
MPKTGLLII	NQVMPKTGLLIIVLA	195	0.0019				-0.0032				1944
LLMQDLVQE	PRKLLMQDLVQENYL	244									1945
FPDLESEFQ	PRMFPDLESEFQAAI	97									1946
ISRKMVELV	QAAISRKMVELVHFL	108									1947
FPVIFSKAS	QDFFPVIFSKASEYL	146									1948
VQENYLEYR	QDLVQENYLEYRQVP	250									1949
FGIEVVEVV	QLVFGIEVVEVVPIS	161				0.0072					1950
IETSYVKVL	RALIETSYVKVLHHT	278									1951
VTKAEMLES	REPVTKAEMLESVLR	129									1952
LMQDLVQEN	RKLLMQDLVQENYLE	245				0.1500					1953
YILVTCLGL	SHLYILVTCLGLSYD	175									1954
LVEVTLGEV	SSTLVEVTLGEVPAA	44									1955
LIIVLAIIA	TGLLIIVLAIIAIEG	201	0.0008				-0.0032				1956
VHFLLLKYR	VELVHFLLLKYRARE	116									1957
VPISHLYIL	VEVVPISHLYILVTC	169									1958
IEVVEVVPI	VFGIEVVEVVPISHL	163									1959
ISHLYILVT	VVPISHLYILVTCLG	171									1960
LSMLEVFEG	WEELSMLEVFEGRED	224									1961
LWGPRALIE	YEFLWGPRALIETSY	271									1962

Table XIX A

Mage 2 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LVGAQAPAT	ALGLVGAQAPATEEQ		-0.0011				1913
LSYDGLLGD	CLGLSYDGLLGDNQV						1914
LGDNQVMPK	DGLLGDNQVMPKTGL		-0.0011				1915
IWEELSMLE	EEKIWEELSMLEVFE						1916
WGPRALIET	EFLWGPRALIETSYV						1917
WEELSMLEV	EKIWEELSMLEVFEG						1918
LEYRQVPGS	ENYLEYRQVPGSDPA						1919
ISYPPLHER	EPHISYPPLHERALR		-0.0011				1920
FQAAISRKM	ESEFQAAISRKMVEL	0.0067	0.5100	0.0310			1921
LGEVPAADS	EVTLGEVPAADSPSP						1922
VIFSKASEY	FFPVIFSKASEYLQL						1923
IFSKASEYL	FPVIFSKASEYLQLV						1924
LGLVGAQAP	GEALGLVGAQAPATE						1925
VVEVVPISH	GIEVVEVVPISHLYI	0.0710	0.0900	0 0089			1926
HVLAHAI	GLLHVLAHAIEGD		-0.0011				1927
LLKYRAREP	HFLLLKYRAREPVTK						1928
HLVTCLGLS	HLYILVTCLGLSYDG						1929
VEVVPISHL	IEVVEVVPISHLYIL						1930
IEGDCAPEE	IIAIEGDCAPEEKIW						1931
LAHAIEGD	IIVLAHAIEGDCAP						1932
LYILVTCLG	ISHLYILVTCLGLSY						1933
MLESVLRNC	KAEMLESVLRNCQDF						1934
LLHVLAH	KTGLLHVLAHAIE	0 0015	0.0290	-0 0004			1935
VPAADSPSP	LGEVPAADSPSPPHS		-0 0011				1936
VGAQAPATE	LGLVGAQAPATEEQQ						1937
VLAHAIEG	LHVLAHAIEGDCA		0 0120				1938
IVLAHAIE	LLHVLAHAIEGDC		0.0130				1939
YRAREPVTK	LLKYRAREPVTKAEM						1940
VFGIEVVEV	LQLVFGIEVVEVVPI						1941
VTLGEVPAA	LVEVTLGEVPAADSP						1942
LVHFLLLKY	MVELVHFLLLKYRAR						1943
MPKTGLLH	NQVMPKTGLLHVLA		-0.0011				1944
LLMQDLVQE	PRKLLMQDLVQENYL						1945
FPDLESEFQ	PRMFPDLESEFQAAI						1946
ISRKMVELV	QAAISRKMVELVHFL						1947
FPVIFSKAS	QDFFPVIFSKASEYL						1948
VQENYLEYR	QDLVQENYLEYRQVP						1949
FGIEVVEVV	QLVFGIEVVEVVPIS						1950
IETSYVKVL	RALIETSYVKVLHHT						1951
VTKAEMLES	REPVTKAEMLESVLR						1952
LMQDLVQEN	RKLLMQDLVQENYLE						1953
YILVTCLGL	SHLYILVTCLGLSYD						1954
LVEVTLGEV	SSTLVEVTLGEVPAA						1955
LHVLAHA	TGLLHVLAHAIEG		-0 0011				1956
VHFLLLKYR	VELVHFLLLKYRARE						1957
VPISHLYIL	VEVVPISHLYILVTC						1958
IEVVEVVPI	VFGIEVVEVVPISHL						1959
ISHLYILVT	VVPISHLYILVTCLG						1960
LSMLEVFEG	WEELSMLEVFEGRED						1961
LWGPRALIE	YEFLWGPRALIETSY						1962

Table XIX A

Mage 2 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2wß1	DR2w2ß2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
VTCLGLSYD	YILVTCLGLSYDGLL	178									1963
LHERALREG	YPPLHERALREGEE-	303									1964
VPGSDPACY	YRQVPGSDPACYEFL	260									1965
VLHHTLKIG	YVKVLHHTLKIGGEP	285									1966

Table XIX A

Mage 2 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VTCLGLSYD	YILVTCLGLSYDGLL						1963
LHERALREG	YPPLHERALREGEE-						1964
VPGSDPACY	YRQVPGSDPACYEFL						1965
VLHHTLKIG	YVKVLHHTLKIGGEP						1966

Table XIX B

Mage 3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2wß1	DR2w2ß2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
VIIFLLLKYR	AELVIIFLLLKYRARE	116									1967
LIIVLAIIA	AGLLIIVLAIIAREG	201	0 0045				-0.0008				1968
LVGAQAPAT	ALGLVGAQAPATEEQ	24	0.0330				-0.0032				1969
LSYDGLLGD	CLGLSYDGLLGDNQI	183				-0.0025					1970
LGDNQIMPK	DGLLGDNQIMPKAGL	189	-0.0003				-0.0032				1971
IWEELSVLE	EEKIWEELSVLEVFE	220				0.0058					1972
WGPRALVET	EFLWGPRALVETSYV	272									1973
WEELSVLEV	EKIWEELSVLEVFEG	221									1974
LEYRQVPGS	ENYLEYRQVPGSDPA	255									1975
FQAALSRKV	ESEFQAALSRKVAEL	104	1.9000	0.3100	1 1000	0.0059	0.0590		0.0310		1976
LGEVPAAES	EVTLGEVPAAESPDP	49									1977
VIFSKASSS	FFPVIFSKASSSLQL	148									1978
IFSKASSSL	FPVIFSKASSSLQLV	149									1979
LGLVGAQAP	GEALGLVGAQAPATE	22									1980
YIFATCLGL	GIILYIFATCLGLSYD	175	0.0110				0 0110				1981
LMEVDPIGH	GIELMEVDPIGHLYI	165									1982
IIVLAIIAR	GLLIIVLAIIAREGD	202									1983
ISYPPLHEW	GPIIISYPPLHEWVLR	298	0.0022				-0.0027				1984
LLKYRAREP	HFLLLKYRAREPVTK	120									1985
IFATCLGLS	HLYIFATCLGLSYDG	176									1986
MEVDPIGHL	IELMEVDPIGHLYIF	166	0 0003	0.0057	-0 0010	1.8000	-0.0055		-0.0008		1987
LYIFATCLG	IGHLYIFATCLGLSY	174									1988
MLGSVVGNW	KAEMLGSVVGNWQYF	134									1989
LLIIVLAII	KAGLLIIVLAIIARE	200	0.0043				-0.0008				1990
LTQHFVQEN	KKLLTQHFVQENYLE	245									1991
VPAAESPDP	LGEVPAAESPDPPQS	52									1992
VGAQAPATE	LGLVGAQAPATEEQE	25									1993
VLAIIAREG	LIIVLAIIAREGDCA	204									1994
IVLAIIARE	LLIIVLAIIAREGDC	203	0 0026				-0.0008				1995
YRAREPVIK	LLKYRAREPVTKAEM	123									1996
VFGIELMEV	LQLVFGIELMEVDPI	160	0.0250	0 0020	0 0013	0 0021	-0 0032		-0.0005		1997
VTLGEVPAA	LVEVTLGEVPAAESP	47									1998
MPKAGLLII	NQIMPKAGLLIIVLA	195	0 0440				-0 0032				1999
YFFPVIFSK	NWQYFFPVIFSKASS	144	0.1100	0.0030	0 0300	0.0006	0.1100		0 0650		2000
FPDLESEFQ	PSTFPDLESEFQAAL	97									2001
FSKASSSLQ	PVIFSKASSSLQLVF	150	0.0510	0.0170	-0 0007	0.0006	0 0240		-0 0005		2002
LSRKVAELV	QAALSRKVAELVIIFL	108									2003
VQENYLEYR	QIIFVQENYLEYRQVP	250									2004
FGIELMEVD	QLVFGIELMEVDPIG	161				0 0150					2005
FPVIFSKAS	QYFFPVIFSKASSSL	146									2006
VETSYVKVL	RALVETSYVKVLIIIM	278									2007
VTKAEMLGS	REPVTKAEMLGSVVG	129									2008
LVEVTLGEV	SSTLVEVTLGEVPAA	44									2009
LVIIFLLLKY	VAELVIIFLLLKYRAR	115									2010
IGHLYIFAT	VDPIGHLYIFATCLG	171									2011
IELMEVDPI	VFGIELMEVDPIGHL	163									2012
WQYFFPVIF	VGNWQYFFPVIFSKA	142									2013
LSVLEVFEG	WEELSVLEVFEGRED	224									2014
LWGPRALVE	YEFLWGPRALVETSY	271									2015
LIIEWVLREG	YPPLHEWVLREGEE-	303									2016

Table XIX B

Mage 3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VHFLLLKYR	AELVHFLLLKYRARE						1967
LHVLAHA	AGLLHVLAHAREG		-0.0026				1968
LVGAQAPAT	ALGLVGAQAPATEEQ		-0.0011				1969
LSYDGLLGD	CLGLSYDGLLGDNQI						1970
LGDNQIMPK	DGLLGDNQIMPKAGL		-0 0011				1971
IWEELSVLE	EEKIWEELSVLEVFE						1972
WGPRALVET	EFLWGPRALVETSYV						1973
WEELSVLEV	EKIWEELSVLEVFEG						1974
LEYRQVPGS	ENYLEYRQVPGSDPA						1975
FQAALSRKV	ESEFQAALSRKVAEL	0.0005	0.7400	0.0430			1976
LGEVPAAES	EVTLGEVPAAESPDP						1977
VIFSKASSS	FFPVIFSKASSSLQL						1978
IFSKASSSL	FPVIFSKASSSLQLV						1979
LGLVGAQAP	GEALGLVGAQAPATE						1980
YIFATCLGL	GHLYIFATCLGLSYD		0.0025				1981
LMEVDPIGH	GIELMEVDPIGHLYI						1982
HVLAHAR	GLLHVLAHAREGD						1983
ISYPPLHEW	GPHISYPPLHEWVLR		-0.0018				1984
LLKYRAREP	HFLLLKYRAREPVTK						1985
IFATCLGLS	HLYIFATCLGLSYDG						1986
MEVDPIGHL	IELMEVDPIGHLYIF	0 0130	0.0027	0 0130			1987
LYIFATCLG	IGHLYIFATCLGLSY						1988
MLGSVVGNW	KAEMLGSVVGNWQYF						1989
LLHVLAH	KAGLLHVLAHARE		-0.0011				1990
LTQHFVQEN	KKLLTQHFVQENYLE						1991
VPAAESPDP	LGEVPAAESPDPPQS						1992
VGAQAPATE	LGLVGAQAPATEEQE						1993
VLAHAREG	LHVLAHAREGDCA						1994
IVLAHARE	LLHVLAHAREGDC		-0.0018				1995
YRAREPVTK	LLKYRAREPVTKAEM						1996
VFGIELMEV	LQLVFGIELMEVDPI	0 0004	0.0970	-0 0004			1997
VTLGEVPAA	LVEVTLGEVPAAESP						1998
MPKAGLLH	NQIMPKAGLLHVLA		-0 0011				1999
YFFPVIFSK	NWQYFFPVIFSKASS	-0.0003	0 0560	0.2200			2000
FPDLESEFQ	PSTFPDLESEFQAAL						2001
FSKASSSLQ	PVIFSKASSSLQLVF	0.0240	0.0890	0 0038			2002
LSRKVAELV	QAALSRKVAELVHFL						2003
VQENYLEYR	QHFVQENYLEYRQVP						2004
FGIELMEVD	QLVFGIELMEVDPIG						2005
FPVIFSKAS	QYFFPVIFSKASSSL						2006
VETSYVKVL	RALVETSYVKVLHHM						2007
VTKAEMLGS	REPVTKAEMLGSVVG						2008
LVEVTLGEV	SSTLVEVTLGEVPAA						2009
LVHFLLLKY	VAELVHFLLLKYRAR						2010
IGHLYIFAT	VDPIGHLYIFATCLG						2011
IELMEVDPI	VFGIELMEVDPIGHL						2012
WQYFFPVIF	VGNWQYFFPVIFSKA						2013
LSVLEVFEG	WEELSVLEVFEGRED						2014
LWGPRALVE	YEFLWGPRALVETSY						2015
LHEWVLREG	YPPLHEWVLREGEE-						2016

Table XIX B

Mage 3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2wß1	DR2w2ß2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
VPGSDPACY	YRQVPGSDPACYEFL	260									2017
VLHHMVKIS	YVKVLHHMVKISGGP	285									2018

Table XIX B

Mage 3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VPGSDPACY	YRQVPGSDPACYEFL						2017
VLHHMVKIS	YVKVLHHMVKISGGP						2018

Table XXa.A
Mage 2 DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LSYDGLLGD	CLGLSYDGLLGDNQV	183				0.1400					2019
IWEELSMLE	EEKIWEELSMLEVFE	220				0 0130					2020
LESEFQAAI	FPDLESEFQAAISRK	100				0.0033					2021
MFPDLESEF	GPRMFPDLESEFQAA	96				0.0890					2022
IEGDCAPEE	IIAIEGDCAPEEKIW	210				0.0660					2023
IAIEGDCAP	LAIIAIEGDCAPEEK	208				0.0190					2024
LVQENYLEY	MQDLVQENYLEYRQV	249				0.2000					2025
FGIEVVEVV	QLVFGIEVVEVVPIS	161				0 0072					2026
LMQDLVQEN	RKLLMQDLVQENYLE	245				0.1500					2027
LLGDNQVMP	YDGLLGDNQVMPKTG	188				0.0270					2028

Table XXa A

Mage 2 DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LSYDGLLGD	CLGLSYDGLLGDNQV						2019
IWEELSMLE	EEKIWEELSMLEVFE						2020
LESEFQAAI	FPDLESEFQAAISRK						2021
MFPDLESEF	GPRMFPDLESEFQAA						2022
IEGDCAPEE	IIAIEGDCAPEEKIW						2023
IAIEGDCAP	LAIIAIEGDCAPEEK						2024
LVQENYLEY	MQDLVQENYLEYRQV						2025
FGIEVVEVV	QLVFGIEVVEVVPIS						2026
LMQDLVQEN	RKLLMQDLVQENYLE						2027
LLGDNQVMP	YDGLLGDNQVMPKTG						2028

Table XXIa B

Mage 3 DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LSYDGLLGD	CLGLSYDGLLGDNQI	183				-0.0025					2029
IWEELSVLE	EEKIWEELSVLEVFE	220				0.0058					2030
LESEFQAAL	FPDLESEFQAALSRK	100				0.0026					2031
MEVDPIGHL	IELMEVDPIGHLYIF	166	0.0003	0.0057	-0.0010	1.8000	-0.0055		-0.0008		2032
IAREGDCAP	LAHAREGDCAPEEK	208				-0.0025					2033
FGIELMEVD	QLVFGIELMEVDPIG	161				0.0150					2034
FVQENYLEY	TQHFVQENYLEYRQV	249				0.2800					2035
LLGDNQIMP	YDGLLGDNQIMPKAG	188				0.0080					2036

Table XXaB
Mage 3 DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LSYDGLLGD	CLGLSYDGLLGDNQI						2029
IWEELSVLE	EEKIWEELSVLEVFE						2030
LESEFQAAL	FPDLESEFQAALSRK						2031
MEVDPIGHL	IELMEVDPIGHLYIF	0.0130	0.0027	0.0130			2032
IAREGDCAP	LAIIAREGDCAPEEK						2033
FGIELMEVD	QLVFGIELMEVDPIG						2034
FVQENYLEY	TQHFVQENYLEYRQV						2035
LLGDNQIMP	YDGLLGDNQIMPKAG						2036

Table XXb A

Mage 2 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
AAISRKMVE	EFQAAISRKMVELVH						2037
MPLEQRSQH	MPLEQRSQHCKP						2038
IGGEPHISY	TLKIGGEPHISYPPL						2039
LHIITLKIGG	VKVLHHTLKIGGEPH						2040

Table XXbA

Mage 2 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
AAISRKMVE	EFQAAISRKMVELVH	106				0.0039					2037
MPLEQRSQH	MPLEQRSQHCKP	1									2038
IGGEPHISY	TLKIGGEPHISYPPL	292				-0.0025					2039
LHITLKIGG	VKVLHHTLKIGGEPH	286				-0.0025					2040

Table XXb B

Mage 3 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
ILGDPKKLL	EDSILGDPKKLLTQH	237	0.0003	-0.0006	-0.0010	0.6700	-0.0055		-0.0008		2041
AALSRKVAE	EFQAALSRKVAELVH	106				0.0027					2042
MPLEQRSQH	MPLEQRSQHCKP	1									2043

Table XXb B

Mage 3 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
ILGDPKKLL	EDSILGDPKKLLTQH	0.0130	-0.0014	0.0029			2041
AALSRKVAE	EFQAALSRKVAELVH						2042
MPLEQRSQH	MPLEQRSQHCKP						2043

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

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Table XXII. A2 supermotif analogs

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
MAGE3.112	9	KVAELVHFL	69	29	14	168	17	5
MAGE3.112L2	9	KLAELVHFL	20	6.0	5.9	12	400	5
MAGE3.112M2	9	KMAELVHFL	24	6.7	7.7	26	286	5
MAGE3.112L2V9	9	KLAELVHFV	14	13	22	15	73	5
MAGE3.112M2V9	9	KMAELVHFV	26	17	46	39	170	5
MAGE3.220	9	KIWEELSVL	333	391	2381	308	--	3
MAGE3.220L2V9	9	KLWEELSVV	11	165	20	15	--	4

-- indicates binding affinity =10,000nM.

Table XXIIA A01 Analog Peptides

Peptide	AA	Sequence	Source	A*0101 nM
52.0026	8	ATCLGLSY	MAGE3.179	227.3
52.013	11	VVEVVPISHLY	MAGE2.166	125
52.0132	11	TMNYPLWSQSY	MAGE3.74	301.2
52.0133	11	LMEVDPIGHLY	MAGE3.166	3.3
57.0003	8	VTDLGLSY	MAGE2.179.D3	2.7
57.0029	9	STFSTTINY	MAGE2.69.T2	490.2
57.003	9	MTDLVQENY	MAGE2.247.T2	0.8
57.0031	9	STLPTTMNY	MAGE3.69.T2	58.1
57.0032	9	GTVVGNWQY	MAGE3.137.T2	36.2
57.0033	9	ETDPIGHLY	MAGE3.168.T2	0.7
57.0034	9	ITGGPHISY	MAGE3.293.T2	36.2
57.0119	10	ATSFSTTINY	MAGE2.68.T2	454.5
57.012	10	ASDFSTTINY	MAGE2.68.D3	25
57.0121	10	LTQDLVQENY	MAGE2.246.T2	58.1
57.0122	10	ATSLPTTMNY	MAGE3.68.T2	208.3
57.0123	10	ASDLPTTMNY	MAGE3.68.D3	2.6
57.0124	10	LTDHFVQENY	MAGE3.246.D3	2.3

Table XXIIB A03 Analog Peptides

Peptide	AA	Sequence	Source	A*0301 nM	A*1101 nM	A*3101 nM	A*3301 nM	A*6801 nM	A3 XRN
1371.63	9	SVFSTTINK	MAGE2.69.V2K9	20	8.2	3333.3	9666.7	5.7	3
1371.64	9	SVFSTTINR	MAGE2.69.V2R9	57.9	6.3	62.1	87.9	6.7	5
1371.65	9	TVINYTLWR	MAGE2.73.V2	261.9	76.9	720	432.8	14.5	4
1371.66	9	TVINYTLWK	MAGE2.73.V2K9	305.6	96.8	9000	-58000	61.5	3
1371.68	9	LVHFLLLKR	MAGE2/3.116.R9	440	375	236.8	93.5	26.7	5
1371.69	9	YVFPVIFSK	MAGE3.138.V2	24.4	3	2769.2	783.8	1.7	3
1371.7	9	YVFPVIFSR	MAGE3.138.V2R9	35.5	2.6	6	13.2	0.5	5
1371.71	8	SVFAHPRR	MAGE2.237.R8	687.5	1538.5	620.7	580	156.9	1
1371.72	9	AVIETSYVK	MAGE2.277.V2	392.9	62.5	12857.1	-290000	30.8	3
1371.73	9	AVIETSYVR	MAGE2.277.V2R9	36666.7	171.4	128.6	1160	15.4	3
1371.74	9	IVYPPLHER	MAGE2.299.V2	117	375	94.7	32.2	13.8	5
1371.75	9	IVYPPLHEK	MAGE2.299.V2K9	42.3	103.4	857.1	2989.7	42.1	3

Table XXIIC A24 Analog Peptides

Peptide	AA	Sequence	Source	A*2401 nM
52.0072	8	LWGPRALI	MAGE2.272	100
52.0073	8	QYFFPVIF	MAGE3.144	100
52.0078	8	SYPPLHEW	MAGE3.300	285.7
52.0102	10	SYPPLHEWVL	MAGE3.300	20.3
52.0166	11	SFSTTINYTLW	MAGE2.70	428.6
52.0167	11	IFSKASEYLQL	MAGE2.150	126.3
52.017	11	IFSKASSSLQL	MAGE3.150	131.9
52.0172	11	IWEELSVLEVF	MAGE3.221	461.5
57.006	9	MYPDLESEF	MAGE2.97.Y2	52.2
57.0061	9	KYVELVHFF	MAGE2.112.Y2F9	7.1
57.0062	9	IYSKASEYF	MAGE2.150.Y2F9	14.6
57.0063	9	EYLQLVFGF	MAGE2.156.F9	4
57.0064	9	VYPKTGLLF	MAGE2.195.Y2F9	5.5
57.0065	9	TYPDLESEF	MAGE3.97.Y2	218.2
57.0066	9	NYQYFFPVF	MAGE3.142.Y2F9	3.4
57.0067	9	IYSKASSSF	MAGE3.150.Y2F9	375
57.0068	9	IYPKAGLLF	MAGE3.195.Y2F9	9.2
57.0084	10	SYSTTINYTF	MAGE2.70.Y2F10	14.8
57.0085	10	LYILVTCLGF	MAGE2.175.F10	17.6
57.0086	10	VYPKTGLLIF	MAGE2.195.Y2F10	2.9
57.0087	10	EYLWGPRALF	MAGE2.270.Y2F10	10
57.0088	10	SYVKVLHHTF	MAGE2.282.F10	34.3
57.009	10	NYQYFFPVIF	MAGE3.142.Y2	22.6
57.0092	10	LYIFATCLGF	MAGE3.175.F10	10
57.0093	10	IYPKAGLLIF	MAGE3.195.Y2F10	1.2
57.0095	10	SYPPLHEWVF	MAGE3.300.F10	5.5

Table XXIII. Immunogenicity of A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound	CTL Wild-type [1]	CTL Tumor
MAGE2.11	9	KMVELVHFL	9.8	25	17	123	2353	4	1/1	0/1
MAGE2.11	10	KMVELVHFLL	23	39	127	9.0	2667	4	1/1	0/1
MAGE2.11	11	KMVELVHFLLL	5.0	45	63	109	7692	4	1/1	0/1
MAGE2.15	9	KASEYLQLV	152	116	17	185	4878	4	2/4	0/2
MAGE2.15	10	YLQLVFGIEV	50	165	345	370	9302	4	3/3	1/3
MAGE2.16	10	LVFGIEVVEV	357	20	43	28	8.0	5	4/4	0/3
MAGE3.11	9	KVAELVHFL	68	29	14	168	17	5	3/4	3/4
MAGE3.11	10	KVAELVHFLL	54	36	217	206	11	5	0/1	0/1
MAGE3.15	11	QLVFGIELMEV	7.9	74	217	185	267	5	3/3	1/3[2]
MAGE3.16	10	LVFGIELMEV	29	20	7.7	28	14	5	4/4	1/4[2]
MAGE3.19	11	IMPKAGLLIIV	20	226	14	176	--[3]	4	3/4	0/3
MAGE3.22	9	KIWEELSVL	357	391	2381	308	--	3	3/4	0/3
MAGE3.27	9	FLWGPRALV	31	43	14	336	40	5	4/4	2/4

1) Indicates the number of donors positive over the total number of donors tested.
2) A positive result was seen after the second restim.
3) -- indicates binding affinity =10,000nM.

Table XXIV. MHC-peptide binding assays: cell lines and radiolabeled ligands.

A. Class I binding assays

Species	Antigen	Allele	Cell line	Radiolabeled peptide	
				Source	Sequence
Human	A1	A*0101	Steinlin	Hu. J chain 102-110	YTAVVPLVY
	A2	A*0201	JY	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0202	P815 (transfected)	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0203	FUN	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0206	CLA	HBVc 18-27 F6->Y	FLPSDYFPSV
	A2	A*0207	721.221 (transfected)	HBVc 18-27 F6->Y	FLPSDYFPSV
	A3		GM3107	non-natural (A3CON1)	KVFPYALINK
	A11		BVR	non-natural (A3CON1)	KVFPYALINK
	A24	A*2402	KAS116	non-natural (A24CON1)	AYIDNYNKF
	A31	A*3101	SPACH	non-natural (A3CON1)	KVFPYALINK
	A33	A*3301	LWAGS	non-natural (A3CON1)	KVFPYALINK
	A28/68	A*6801	C1R	HBVc 141-151 T7->Y	STLPETYVVRR
	A28/68	A*6802	AMAI	HBV pol 646-654 C4->A	FTQAGYPAL
	B7	B*0702	GM3107	A2 sigal seq. 5-13 (L7->Y)	APRTLVYLL
	B8	B*0801	Steinlin	HIVgp 586-593 Y1->F, Q5->Y	FLKDYQLL
	B27	B*2705	LG2	R 60s	FRYNGLIHR
	B35	B*3501	C1R, BVR	non-natural (B35CON2)	FPFKYAAAF
	B35	B*3502	TISI	non-natural (B35CON2)	FPFKYAAAF
	B35	B*3503	EHM	non-natural (B35CON2)	FPFKYAAAF
	B44	B*4403	PITOUT	EF-1 G6->Y	AEMGKYSFY
	B51		KAS116	non-natural (B35CON2)	FPFKYAAAF
	B53	B*5301	AMAI	non-natural (B35CON2)	FPFKYAAAF
	B54	B*5401	KT3	non-natural (B35CON2)	FPFKYAAAF
	Cw4	Cw*0401	C1R	non-natural (C4CON1)	QYDDAVYKL
	Cw6	Cw*0602	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL
	Cw7	Cw*0702	721.221 transfected	non-natural (C6CON1)	YRHDGGNVL
Mouse	D^b		EL4	Adenovirus E1A P7->Y	SGPSNTYPEI
	K^b		EL4	VSV NP 52-59	RGYVFQGL
	D^d		P815	HIV-IIIB ENV G4->Y	RGPYRAFVTI
	K^d		P815	non-natural (KdCON1)	KFNPMKTYI
	L^d		P815	HBVs 28-39	IPQSLDSYWTSL

B. Class II binding assays

Species	Antigen	Allele	Cell line	Radiolabeled peptide	
				Source	Sequence
Human	DR1	DRB1*0101	LG2	HA Y307-319	YPKYVKQNTLKLAT
	DR2	DRB1*1501	L466.1	MBP 88-102Y	VVHFFKNIVTPRTPPY
	DR2	DRB1*1601	L242.5	non-natural (760.16)	YAAFAAAKTAAAFA
	DR3	DRB1*0301	MAT	MT 65kD Y3-13	YKTIAFDEEARR
	DR4w4	DRB1*0401	Preiss	non-natural (717.01)	YARFQSQTTLKQKT
	DR4w10	DRB1*0402	YAR	non-natural (717.10)	YARFQRQTTLKAAA
	DR4w14	DRB1*0404	BIN 40	non-natural (717.01)	YARFQSQTTLKQKT
	DR4w15	DRB1*0405	KT3	non-natural (717.01)	YARFQSQTTLKQKT
	DR7	DRB1*0701	Pitout	Tet. tox. 830-843	QYIKANSKFIGITE
	DR8	DRB1*0802	OLL	Tet. tox. 830-843	QYIKANSKFIGITE
	DR8	DRB1*0803	LUY	Tet. tox. 830-843	QYIKANSKFIGITE
	DR9	DRB1*0901	HID	Tet. tox. 830-843	QYIKANSKFIGITE
	DR11	DRB1*1101	Sweig	Tet. tox. 830-843	QYIKANSKFIGITE
	DR12	DRB1*1201	Herluf	unknown eluted peptide	EALIHQLKINPYVLS
	DR13	DRB1*1302	H0301	Tet. tox. 830-843 S->A	QYIKANAKFIGITE
	DR51	DRB5*0101	GM3107 or L416.3	Tet. tox. 830-843	QYIKANAKFIGITE
	DR51	DRB5*0201	L255.1	HA 307-319	PKYVKQNTLKLAT
	DR52	DRB3*0101	MAT	Tet. tox. 830-843	NGQIGNDPNRDIL
	DR53	DRB4*0101	L257.6	non-natural (717.01)	YARFQSQTTLKQKT
	DQ3.1	A1*0301/DQB1*0	PF	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
Mouse	IA^b		DB27.4	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA^d		A20	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA^k		CH-12	HEL 46-61	YNTDGSTDYGILQINSR
	IA^s		LS102.9	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IA^u		91.7	non-natural (ROIV)	YAHAAHAAHAAHAAHAA
	IE^d		A20	Lambda repressor 12-26	YLEDARRKKAIYEKKK
	IE^k		CH-12	Lambda repressor 12-26	YLEDARRKKAIYEKKK

Table XXV. Antibodies used in MHC purification.

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 Db and Ld
34-5-8S	H-2 Dd
B8-24-3	H-2 Kb
SF1-1.1.1	H-2 Kd
Y-3	H-2 Kb
10.3.6	H-2 IAk
14.4.4	H-2 IEd, IEK
MKD6	H-2 IAd
Y3JP	H-2 IAb, IAs, IAu

Table XXVI. Crossbinding data A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
MAGE2.112	9	KMVELVHFL	38	15	9.1	49	364	5
MAGE2.112	10	KMVELVHFLL	23	39	127	9.0	2667	4
MAGE2.112	11	KMVELVHFLLL	5.0	45	63	109	7692	4
MAGE2.153	9	KASEYLQLV	152	116	17	185	4878	4
MAGE2.157	10	YLQLVFGIEV	50	165	345	370	9302	4
MAGE2.160	10	LVFGIEVVEV	357	21	44	29	8.0	5
MAGE2.220	9	KIWEELSML	167	642	175	29	--	3
MAGE2.271	9	FLWGPRALI	238	96	137	1542	95	4
MAGE2.277	10	ALIETSYVKV	500	729	125	1947	3077	2
MAGE2/3.44	10	TLVEVTLGEV	67	39	4.3	218	33	5
MAGE3.112	9	KVAELVHFL	68	29	14	168	17	5
MAGE3.112	10	KVAELVHFLL	54	36	217	206	11	5
MAGE3.159	11	QLVFGIELMEV	7.9	74	217	185	267	5
MAGE3.160	10	LVFGIELMEV	29	20	7.7	29	14	5
MAGE3.174	11	HLYIFATCLGL	56	741	769	--	4494	1
MAGE3.176	9	YIFATCLGL	185	45	37	1028	222	4
MAGE3.195	11	IMPKAGLLIIV	20	226	15	176	--	4
MAGE3.220	9	KIWEELSVL	333	391	2381	308	--	3
MAGE3.271	9	FLWGPRALV	31	43	14	336	40	5

-- indicates binding affinity =10,000nM.

Table XXVII. Immunogenicity of A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound	CTL Wild-type [1]	CTL Tumor
MAGE2.11	9	KMVELVHFL	9.8	25	17	123	2353	4	1/1	0/1
MAGE2.11	10	KMVELVHFLL	23	39	127	9.0	2667	4	1/1	0/1
MAGE2.11	11	KMVELVHFLLL	5.0	45	63	109	7692	4	1/1	0/1
MAGE2.15	9	KASEYLQLV	152	116	17	185	4878	4	2/4	0/2
MAGE2.15	10	YLQLVFGIEV	50	165	345	370	9302	4	3/3	1/3
MAGE2.16	10	LVFGIEVVEV	357	20	43	28	8.0	5	4/4	0/3
MAGE3.11	9	KVAELVHFL	68	29	14	168	17	5	3/4	3/4
MAGE3.11	10	KVAELVHFLL	54	36	217	206	11	5	0/1	0/1
MAGE3.15	11	QLVFGIELMEV	7.9	74	217	185	267	5	3/3	1/3[2]
MAGE3.16	10	LVFGIELMEV	29	20	7.7	28	14	5	4/4	1/4[2]
MAGE3.19	11	IMPKAGLLIIV	20	226	14	176	--[3]	4	3/4	0/3
MAGE3.22	9	KIWEELSVL	357	391	2381	308	--	3	3/4	0/3
MAGE3.27	9	FLWGPRALV	31	43	14	336	40	5	4/4	2/4

1) Indicates the number of donors positive over the total number of donors tested.
2) A positive result was seen after the second restim.
3) -- indicates binding affinity =10,000nM.

Table XXVIII. DR supertype primary binding

Peptide	DR147 Algo Sum	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR147 Cross-binding
39.0282	2	LGEVPAADSPSPPHS	MAGE2.50	--	--	--	0
39.0283	3	ESEFQAAISRKMVEL	MAGE2.102	4.2	281	49	3
39.0284	2	GIEVVEVVPISHLYI	MAGE2.163	595	6429	278	2
39.0285	2	DGLLGDNQVMPKTGL	MAGE2.187	--	--	--	0
39.0286	2	NQVMPKTGLLIIVLA	MAGE2.193	2632	--	--	0
39.0287	2	KTGLLIIVLAIIAIE	MAGE2.198	417	1216	862	2
39.0288	2	TGLLIIVLAIIAIEG	MAGE2.199	6250	--	--	0
39.0291	2	GLLIIVLAIIAIEGD	MAGE2.200	500	--	--	1
39.0292	3	LLIIVLAIIAIEGDC	MAGE2.201	581	3750	1923	1
39.0293	2	LIIVLAIIAIEGDCA	MAGE2.202	417	8824	2083	1
39.0294	2	EPHISYPPLHERALR	MAGE2.296	--	--	--	0
39.0295	3	ALGLVGAQAPATEEQ	MAGE2/3.22	152	--	--	1
39.0296	2	ESEFQAALSRKVAEL	MAGE3.102	2.6	763	34	3
39.0297	2	NWQYFFPVIFSKASS	MAGE3.142	46	409	446	3
39.0298	3	PVIFSKASSSLQLVF	MAGE3.148	98	1875	281	2
39.0299	3	LQLVFGIELMEVDPI	MAGE3.158	200	--	258	2
39.0300	3	GHLYIFATCLGLSYD	MAGE3.173	455	4091	--	1
39.0301	2	DGLLGDNQIMPKAGL	MAGE3.187	--	--	--	0
39.0302	2	NQIMPKAGLLIIVLA	MAGE3.193	114	--	--	1
39.0303	2	KAGLLIIVLAIIARE	MAGE3.198	1163	--	--	0
39.0304	2	AGLLIIVLAIIAREG	MAGE3.199	1111	--	>9615	0
39.0305	3	LLIIVLAIIAREGDC	MAGE3.201	1923	--	--	0
39.0306	2	GPHISYPPLHEWVLR	MAGE3.296	2273	--	--	0

-- indicates binding affinity =10,000nM.

Table XXIX. DR supertype crossbinding

Peptide	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR2w2 ß1 nM	DR2w2 ß2 nM	DR6w1 9 nM	DR5w1 1 nM	DR8w2 nM	DR147 Cross-binding	Broad Binding (5/8)
39.0283	ESEFQAAISRKMVEL	MAGE2.102	4.2	281	49	147	20	522	741	1581	3	7
39.0284	GIEVVEVVPISHLYI	MAGE2.163	595	6429	278	1978	--	49	--	5506	2	3
39.0287	KTGLLIIVLAIIAIE	MAGE2.198	417	1216	862	2460	--	2333	--	--	2	2
39.0296	ESEFQAALSRKVAEL	MAGE3.102	2.6	763	34	29	18	7000	645	1140	3	6
39.0297	NWQYFFPVIFSKASS	MAGE3.142	46	409	446	3033	667	--	308	223	3	6
39.0298	PVIFSKASSSLQLVF	MAGE3.148	98	1875	281	535	--	146	--	--	2	4
39.0299	LQLVFGIELMEVDPI	MAGE3.158	200	--	258	4550	--	8750	--	--	2	2

-- indicates binding affinity =10,000nM.

Table XXX. DR3 binding

Peptide	Sequence	Source	DR3 nM
39.0384	GPRMFPDLESEFQAA	MAGE2.94	3371
39.0387	FPDLESEFQAAISRK	MAGE2.98	--
39.0388	EFQAAISRKMVELVH	MAGE2.104	--
39.0389	QLVFGIEVVEVVPIS	MAGE2.159	--
39.0390	CLGLSYDGLLGDNQV	MAGE2.181	2143
39.0391	YDGLLGDNQVMPKTG	MAGE2.186	--
39.0392	LAIIAIEGDCAPEEK	MAGE2.206	--
39.0393	IIAIEGDCAPEEKIW	MAGE2.208	4546
39.0394	EEKIWEELSMLEVFE	MAGE2.218	--
39.0395	RKLLMQDLVQENYLE	MAGE2.243	2000
39.0396	MQDLVQENYLEYRQV	MAGE2.247	1500
39.0397	VKVLHHTLKIGGEPH	MAGE2.284	--
39.0398	TLKIGGEPHISYPPL	MAGE2.290	--
39.0399	FPDLESEFQAALSRK	MAGE3.98	--
39.0400	EFQAALSRKVAELVH	MAGE3.104	--
39.0401	QLVFGIELMEVDPIG	MAGE3.159	--
39.0402	IELMEVDPIGHLYIF	MAGE3.164	167
39.0403	CLGLSYDGLLGDNQI	MAGE3.181	--
39.0404	YDGLLGDNQIMPKAG	MAGE3.186	--
39.0405	LAIIAREGDCAPEEK	MAGE3.206	--
39.0406	EEKIWEELSVLEVFE	MAGE3.218	--
39.0407	EDSILGDPKKLLTQH	MAGE3.235	448
39.0408	TQHFVQENYLEYRQV	MAGE3.247	1071

-- indicates binding affinity =10,000nM.

Table XXXI. HTL Candidates

Peptide	Sequence	Motif	Source	DR1 nM	DR4w4 nM	DR7 nM	DR3 nM	DR2w2 ß1 nM	DR2w2 ß2 nM	DR6w1 9 nM	DR5w1 1 nM	DR8w2 nM	DR147 Cross-binding	Broad Binding (5/8)	DR3 Binder
39.0283	ESEFQAAISRKMVEL	DR sup	MAGE2.102	4.2	281	49	--	147	20	522	741	1581	3	7	0
39.0296	ESEFQAALSRKVAEL	DR sup	MAGE3.102	2.6	763	34	--	29	18	7000	645	1140	3	6	0
39.0297	NWQYFFPVIFSKASS	DR sup	MAGE3.142	46	409	446	--	3033	667	--	308	223	3	6	0
39.0402	IELMEVDPIGHLYIF	DR3	MAGE3.164	--	>8182	9259	167	1597	--	269	--	3769	0	1	1
39.0407	EDSILGDPKKLLTQH	DR3	MAGE3.235	--	>8182	--	448	--	--	269	--	--	0	1	1

-- indicates binding affinity =10,000nM.

WHAT IS CLAIMED IS

1. A peptide composition of less than 500 amino acid residues comprising a peptide epitope useful for inducing an immune response against MAGE2/3 said epitope (a) having an amino acid sequence of about 8 to about 13 amino acid residues that have at least 65% identity with a native amino acid sequence of MAGE2/3 and, (b) binding to at least one HLA class I allele with an IC_{50} of less than about 500 nM.

2. The composition of claim 1, further wherein said peptide has at least 77% identity with a native MAGE2/3 amino acid sequence.

3. The composition of claim 1, further wherein said peptide has 100% identity with a native MAGE2/3 amino acid sequence.

4. A pharmaceutical composition comprising a peptide and a pharmaceutical carrier, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A*0201 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif) comprising an IC_{50} of less than about 500 nM for at least one HLA class I molecule.

5. The pharmaceutical composition of claim 4 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.

6. The pharmaceutical composition of claim 5 wherein the composition comprises the peptide in a form of nucleic acids that encode the epitope and one or more additional peptide(s).

7. The composition of claim 4, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

8. The pharmaceutical composition of claim 4 wherein the peptide is in a human dose form, and the carrier is in a human unit dose.

9. A peptide composition of claim 1 comprising an analog of a peptide epitope, wherein the peptide epitope is an epitope of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), or Table XVIII (A24 motif), said analog comprising a preferred or less preferred amino acid of Table II substituted in for a starting residue, or having a deleterious residue of Table II substituted out of the starting sequence and replaced by a non-deleterious residue.

10. A peptide composition of claim 9 comprising a peptide of Table XXII.

11. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a peptide that comprises an IC_{50} of less than about 500 nM for an HLA class I molecule, wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), Table XVIII (A24 motif), or Table XXII; and,

administering said peptide to a human.

12. The method of claim 11, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

13. The method of claim 12, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

14. The method of claim 11, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

15. A method for inducing a cytotoxic T lymphocyte response, said method comprising steps of:

providing a pharamceutical composition comprising a peptide and a pharmaceutical carrier, wherein the peptide induces a cytotoxic T cell response *in vitro* and/or *in vivo*, and further wherein the peptide is a peptide of Table VII (A1 supermotif), Table VIII (A2 supermotif/A2.1 motif), Table IX (A3 supermotif), Table X (A24 supermotif), Table XI (B7 supermotif), Table XII (B27 supermotif), Table XIII (B58 supermotif), Table XIV (B62 supermotif), Table XV (A1 motif), Table XVI (A3 motif), Table XVII (A11 motif), Table XVIII (A24 motif), or Table XXIII; and,

administering said pharmaceutical composition to a human.

16. The method of claim 15, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

17. The method of claim 16, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

18. The method of claim 15, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

19. The method of claim 15, wherein the providing step comprises a peptide that induces a cytotoxic T cell response when complexed with an HLA class I molecule and is presented to an HLA class I-restricted cytotoxic T cell.

20. A peptide composition of less than 500 amino acid residues comprising a peptide epitope useful for inducing an immune response against MAGE2/3 said epitope (a) having an amino acid sequence of about 6 to about 25 amino acid residues that have at least 65% identity with a native amino acid sequence of MAGE2/3 and, (b) binding to at least one HLA class II HLA allele with an IC_{50} of less than about 1000 nM.

21. The peptide composition of claim 20, further wherein said peptide has at least 77% identity with a native MAGE2/3 amino acid sequence.

22. The peptide composition of claim 20, further wherein said peptide has 100% identity with a native MAGE2/3 amino acid sequence.

23. A pharmaceutical composition comprising:

a human dose form of a peptide of Table XIX or Table XX that comprises an IC_{50} of less than about 1,000 nM for at least one HLA DR molecule of an HLA DR supertype; and,

a human dose of a pharmaceutically acceptable carrier.

24. The pharmaceutical composition of claim 23 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide.

25. The pharmaceutical composition of claim 24 wherein the composition comprises the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

26. The pharmaceutical composition of claim 25, wherein the peptide is comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

27. A peptide composition of claim 20 comprising an analog of a peptide epitope of Table XIX or Table XX, said analog comprising a preferred or less preferred amino acid of Table III substituted in for a starting residue, and/or having a deleterious residue of Table III substituted out of the starting sequence and replaced by a non-deleterious residue.

28. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a pharmaceutical composition comprising a human dose of a peptide that comprises an IC_{50} of less than about 1,000 nM for an HLA class II molecule and a human dose of a pharmaceutical carrier, wherein the peptide is a peptide of Table XIX or Table XX; and,

administering said peptide to a human.

29. The method of claim 28, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

30. The method of claim 29, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

31. The method of claim 28, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

32. A method for inducing a helper T lymphocyte response, said method comprising steps of:

providing a pharmaceutical composition comprising a human dose of a peptide that induces a helper T cell response *in vitro* and/or *in vivo* and a pharmaceutically acceptable carrier, wherein the peptide is a peptide of Table XIX or Table XX; and,

administering said pharmaceutical composition to a human.

33. The method of claim 32, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide.

34. The method of claim 33, wherein the providing step provides the peptide in a form of nucleic acids that encode the peptide and at least one additional peptide, with a *proviso* that an additional peptide is not an entire native antigen.

35. The method of claim 32, wherein the providing step provides the peptide comprised by a longer peptide, with a *proviso* that the longer peptide is not an entire native antigen.

36. The method of claim 32, wherein the providing step comprises a peptide that induces a helper T cell response when complexed with an HLA class II molecule and is presented to an HLA class II-restricted helper T cell.

37. A vaccine for preventing or treating cancer that induces a protective or therapeutic immune response, wherein said vaccine comprises:

at least one peptide selected from Table(s) VII-XX or Table XXII; and,

a pharmaceutically acceptable carrier.

38. A kit for a vaccine that induces a protective or therapeutic immune response to a tumor, said vaccine comprising:

at least one peptide selected from Table(s) VII-XX or Table XXII;

a pharmaceutically acceptable carrier; and,

instructions for administration to a patient.

39. A method for monitoring or evaluating an immune response to a tumor or an epitope thereof in a patient having a known HLA type, the method comprising:

incubating a T lymphocyte sample from the patient with a peptide selected from Table(s) VII-XX or Table XXII, wherein that peptide bears a motif corresponding to at least one HLA allele present in said patient; and,

detecting the presence of a T lymphocyte that recognizes the peptide.

40. The method of claim 39, wherein the peptide is comprised by a tetrameric complex.

ABSTRACT OF THE DISCLOSURE

This invention uses our knowledge of the mechanisms by which antigen is recognized by T cells to identify and prepare MAGE2/3 epitopes, and to develop epitope-based vaccines directed towards MAGE2/3-bearing tumors. More specifically, this application communicates our discovery of pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS** the specification of which __X__ is attached hereto or ___ was filed on _____ as Application No. _____ and was amended on _____ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
09/189,702	November 10, 1998	pending
08/205,713	March 4, 1994	pending
08/159,184	November 29, 1993	abandoned
08/073,205	June 4, 1993	abandoned
08/027,146	March 5, 1993	abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Signature of Inventor 4 SCOTT SOUTHWOOD Date	Signature of Inventor 5 ROBERT CHESNUT Date	Signature of Inventor 6 ESTEBAN CELIS Date
Signature of Inventor 7 ELISSA KEOGH Date		

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